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Research Article

CORRELATION OF ALBUMIN CORRECTED SERUM CALCIUM WITH SEVERITY OF ILLNESS IN DENGUE PATIENTS

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ABSTRACT
Dengue infection is a major public health problem leading to significant morbidity and mortality. In dengue infection, plasma leakage is observed leading to numerous biochemical derangements including hypocalcemia. 117 patients diagnosed with dengue fever, clinically and serologically, were studied. Serum calcium was estimated at the time of admission and discharge. Mean serum calcium level on the day of admission and discharge were 7.63mg/dL and 8.44 mg/dL respectively. In this study we found that, lower the platelet levels, lower was the serum calcium levels, and the results were statistically significant (p value <0.05). Serum calcium level was significantly reduced in majority of patients with dengue infection and can be used as an additional prognostic factor to assess the clinical outcome of the illness.

Keywords: *Calcium, Dengue, Hypocalcemia*

INTRODUCTION
Dengue is a transmittable disease caused by the Dengue virus, which is the member of the genus Flavivirus (Rodenhuis-Zybert, *et al*., 2010), in the family Flaviviridae, with four antigenically distinct serotypes (Dengue 1–4), transmitted to humans by the vector mosquito Aedes aegypti or Aedes albopictus.
Dengue infection primarily occurs in tropical and sub-tropical regions throughout of the world, mainly in urban and semi-urban areas.
Dengue infection is a major public health problem leading to significant morbidity and mortality. In dengue infection, plasma leakage is observed leading to numerous biochemical derangements including hypocalcemia.
The World Health Organization has reported that an estimated 2.5 billion people- two-fifths of the world population live in areas where dengue infection can be transmitted, annually up to 50 million infections arise worldwide, including 500 000 cases of Dengue Hemorrhagic Fever (DHF) and 25,000 deaths. In South East Asia, the average annual figure of DHF cases has increased from 10,000 in the 1950s to more than 200,000 in the 1990s (C. Sirivichayakul,*et al*., 2012, and E. Khan, *et al*., 2010).
In persons infected with dengue virus, extracellular calcium plays an important role in platelet aggregation and for the regulation of the immune response.
Calcium has been shown to be essential for cytotoxic activity of the dengue virus-induced macrophage cytotoxin (CF2) (R. Dhawan,*et al*., 1991).
Calcium appears to play a role in the induction of dengue-specific T-helper cells.

Hypocalcemia in dengue
Several causes for low blood calcium levels have been suggested, including reduced Na+-K adenosine triphosphatase (ATPase) activity, reduced Ca2+-ATPase activity.
Low blood calcium levels have been demonstrated in dengue infection (Bunnag T,*et al*., 2011; Kapoor. S, *et al*., 2012 and Wiwanitkit S,*et al*., 2012) and is often underrecognized but can present with tetany (Wiwanitkit S,*et al*., 2012).There is little information on the other effects of hypocalcemia, although lower calcium levels have not shown an association with mortality (Bunnag T,*et al*., 2011).

The possible role of calcium in the immunopathogenesis of dengue
In in vitro studies, depletion of Mg2+ and Ca2+ has been shown to enhance binding of dengue virus to monocyte macrophages and cells of T cell and B cell lineages (Bielefeldt-Ohmann H,*et al*., 2001). Ca2+

has been shown to be essential for cytotoxic activity of the dengue type 2 virus (DV)-induced macrophage cytotoxin (CF2); cell death has been shown to be associated with increased intracellular Ca2+ (R. Dhawan,*et al*., 1991; Khanna M,*et al*., 1991)**.** Ca2+ appears to play a role in the induction of dengue-specific T-helper cells. Dengue antigen has been shown to increase the influx of Ca2+ into T-cells. The proliferation of dengue-specific T-helper cells appears to be dependent on Ca2+ and is inhibited in the absence of Ca2+ and by calcium channel antagonist drugs (Chaturvedi P,*et al*., 1995).
In another in vitro study, production of DV-induced suppressor cytokine was inhibited when the medium was depleted of calcium; production was restored by addition of calcium to the medium. Both the production of suppressor cytokine and transmission of the suppressor signal were inhibited in a dose-dependent manner by the calcium channel antagonists verapamil and nifedipine (Khare M,*et al*., 1995). There is some evidence that the production of nitrite in response to dengue virus infection is also calcium dependent and can be inhibited by calcium channel blocking drugs (Misra A, *et al*., 1996; Mukerjee R, *et al*., 1996). Thus, calcium appears to play a role in the immune response in dengue, although the interactions are complex, and the precise clinical implications of these interactions are yet not clearly defined.

Calcium and the myocardium in dengue

Calcium plays a key role in the functioning of myocardial tissue. Cardiac involvement in dengue has been documented in many studies, although little is known about its actual pathogenesis (Wichmann D,*et al*., 2009; Kularatne SA, *et al*., 2007)
It has been proposed that the derangements of Ca2+ storage in the infected myocardial cells may directly contribute to the development of myocarditis.
In an in-vitro study, human skeletal myotubes were exposed to dengue virus in vitro and intracellular Ca2+ changes were assayed. Dengue virus capsid proteins were demonstrated in myotubes by confocal immunofluorescence microscopy, confirming virus infection and replication in myotubes. An increase in resting intracellular Ca2+ was demonstrated in infected skeletal myotubes when compared with non-exposed controls. The authors suggested that this increase in resting (i.e. diastolic) Ca2+ levels in infected myocardium may be responsible for arrhythmias and altered contractile function (Salgado DM,*et al*., 2010).

The potential role of calcium in treatment of dengue

Calcium is needed for platelet aggregation, although its precise role is not known (Authi KS, *et al*., 2007;Colomer J, *et al*., 2007)
There is no hard evidence of benefit that calcium supplementation is beneficial in dengue, although the limited evidence suggests that it is an area needing further study (Sanchez-Valdez E, *et al*., 2009; Cabrera-Cortina JI, *et al*., 2008).

Aims and objectives

To study the correlation between serum calcium levels and other clinical and laboratory parameters in patients with dengue fever.

MATERIALS AND METHODS

117 patients aged more than 18 years, diagnosed with dengue fever, clinically and serologically, admitted in MVJMC&RH were included in study.
Patients were evaluated clinically and all relevant investigations were done.
Serum calcium was estimated at the time of admission and discharge.

RESULTS AND DISCUSSION

Most common symptom was fever (100%), followed by myalgia (84.6%), nausea & vomiting (47.8%) and headache (35.9%).42 patients (35.8%) had mucosal bleed and 42 (35.8%) patients had hepatomegaly, 24 patients (20.5%) had bradycardia (Fig. 1).

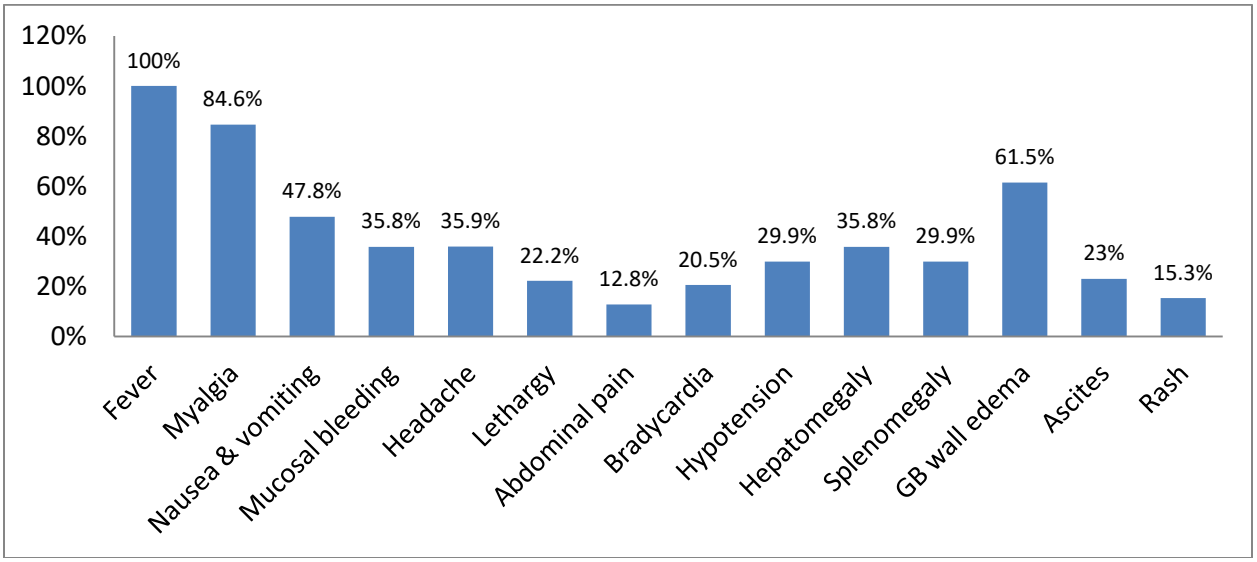

Fig 1: Frequency of assessed parameters in patients with dengue fever:

Mean serum calcium level on date of admission (DOA) among males was 7.72±0.86 mg/dL & among females was 7.52±0.87 mg/dL.On day of discharge (DOD) mean serum calcium level among males was 8.45±0.40 mg/dL whereas it was 8.42±0.40 mg/dL among females.It was observed that the mean serum calcium level on both DOA and DOD was comparatively less among females. And total mean serum calcium levels on DOA was 7.63±0.862 mg/dL and on DOD was 8.44±0.399 mg/dL (Table 1).

Table 1: Number of patients with Serum Calcium (mean) levels

Serum calcium (mg/dL) (Mean)	**Male (n=70)**	**Female (n=47)**	**Total**	**P value**
DOA	7.72±0.86	7.52±0.87	**7.63±0.862**	**<0.0001**
DOD	8.45±0.40	8.42±0.40	**8.44±0.399**	

Serum calcium did not correlate with the fever, hepatosplenomegaly or ascites.Whereas correlation between serum calcium and bradycardia was statistically significant with a p value of 0.026 (Table2).

Table 2: Correlation of clinical features with serum calcium:

Clinical features	Serum calcium (mg/dL)			P value
	>6 ≤7	>7 ≤8	>8 ≤9	
Fever	16	9	10	0.073
Bradycardia (<60bpm)	3	13	8	**0.026**
Hepatomegaly	12	19	11	0.1
Splenomegaly	13	12	10	0.078
Ascites	8	10	9	0.905

As we observe from this chart, we find that lower the platelet count, lower was the serum calcium levels.And the correlation between serum calcium level and platelet count was statistically significant with a p value of< 0.0001 (Table 3).

Table 3: Correlation of serum calcium with platelet count:

Platelets	Serum calcium (mg/dL)			Total	p value
	>6 ≤7	>7 ≤8	>8 ≤9		
≤50,000	**28**	3	2	33	**<0.0001**
50,000-1L	5	**30**	5	40	
1-1.5	3	4	**16**	23	
≥1.5	0	2	**19**	21	
	36	39	42	117	

88 patients out of 117 patients were NS1 +ve, and were significantly correlating with the serum calcium levels.As the patient started recovering, i.e., patients with IgG +ve had serum calcium levels >8mg/dL.

Hence IgG correlated significantly with serum calcium levels. Whereas there was no statistically significant difference between IgM and serum calcium levels (Table 4).

Table 4: Correlation of dengue serology with Serum calcium

Serology	**Serum calcium (mg/dL)**			**P value**
	>6 ≤7	>7 ≤8	>8 ≤9	
NS1(+)	30	32	26	**0.044**
IgM	18	20	27	0.362
IgG	1	5	14	**0.001**

36 out of 117 patients on the DOA had serum calcium level of 6-7mg/dL, 39 patients between 7-86-7mg/dL and 42 patients had calcium levels of 8-96-7mg/dL.
While on the DOD 95 patients out of 117 had normal serum calcium levels i.e.,>8mg/dL (Table 5).

Table 5: Comparison between number of patients on day of admission and day of discharge and Serum Calcium:

Serum calcium (mg/dL)	**DOA** (No.)	**DOD** (No.)
>6 ≤7	36	0
>7 ≤8	39	22
>8 ≤9	42	95

We observe that as the serum calcium levels are lower, the duration of hospital stay increases. And when the calcium levels are within normal range the duration of hospital stay was found to be < 5days.This difference was statistically significant with a p value of 0.011 (Table 6).

Table 6: Correlation of duration of hospital stay with serum calcium

Duration of hospital stay (days)	**Serum calcium (mg/dL)**			**P value**
	>6 ≤7	>7 ≤8	>8 ≤9	
<5	06	10	19	0.011
6-10	12	17	16	
11-15	18	12	07	

DISCUSSION

In our study, out of 117 patients, males were 70(59.9%) and females were 47(40.1%).

Most common symptom was fever (100%), followed by myalgia (84.6%), nausea & vomiting(47.8%) and headache(35.9%).

42 patients (35.8%) had bleeding manifestations in the form of mucosal bleed and 42 (35.8%) patients had hepatomegaly, 24 patients(20.5%) had bradycardia.

Mean serum calcium level on the day of admission and discharge were 7.63±0.862 mg/dL and 8.44±0.399 mg/dL respectively. This implies that mean serum calcium levels were less on day of admission (during illness) and became normal on the day of discharge; once the illness subsides.

In this study we found that, lower the platelet levels, lower was the serum calcium levels, and the results were statistically significant (p value <0.05).

41 (35%) patients had platelet transfusion, out of which 17(41.4%) patients had a serum calcium of 6-7mg/dL, 16 (39%) patients had a serum calcium of 7-8mg/dL & 8 (19.6%) patients had serum calcium of 8-9mg/dL, which indirectly tells that lesser serum calcium levels, increase in the platelet transfusion rate.

Low serum calcium levels correlated significantly with:

1. severity of dengue illness
2. increased risk of bleeding manifestations,
3. increased number of platelet transfusion and
4. the duration of the hospital stay.

There was no mortality in this study.

In our study it was found that calcium level were decreased significantly as compared to control. The observed significant values of calcium in dengue patients correlate with findings of Castilla-Guerra *et al*, and Sara Syed, *et al*, which stated that acute hypocalcemia is primary cause of increased neuromuscular

excitability and tetany is frequently associated with dengue virus infection (L.Castilla-Guerra, *et al*., 2006; Sara Syed, *et al*., 2014).

CONCLUSION

Serum calcium level was significantly reduced in majority of patients with dengue infection and can be used as an additional prognostic factor to assess the clinical outcome of the illness.
We recommend carrying out prospective studies to examine use of serum calcium level as predictor for the severity of illness and carrying out randomized controlled studies on oral and intravenous calcium replacement for dengue fever patients to assess its role in prevention of complications and clinical recovery.

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