*Brief Original Article*

# Hypocalcemia is associated with disease severity in patients with dengue

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## Abstract

Introduction: Dengue hemorrhagic fever (DHF) is a major cause of morbidity and mortality in tropical regions. Serum free calcium ($Ca^{2+}$) is known to be important in cardiac and circulatory function. We evaluated association between serum $Ca^{2+}$ level and severity of dengue.

Methodology:A cross-sectional study was carried out at a tertiary care private hospital in Sri Lanka. A probable case of dengue was diagnosed and classified according to World Health Organization criteria and confirmed by either IgM antibody, PCR, or NS1 antigen detection. Socio-demographic details were collected using an interviewer-administered questionnaire.

Results: The sample size was 135. The mean age was 26.1 years, and the majority were males (n = 80, 59.3%). DHF was diagnosed in 71 patients (52.6%). Mean serum $Ca^{2+}$ level of the study population was 1.05 mmol/L (range 0.77–1.24). Mean serum $Ca^{2+}$ was significantly higher in patients with dengue fever (DF) (1.09 mmol/L) than in those with DHF (1.02 mmol/L) ($p < 0.05$). A significant difference was observed between mean serum calcium levels of DHF I and DHF II. Prevalence of hypocalcemia in DHF and DF patients was 86.9% (n = 60) and 29.7% (n = 11), respectively ($p < 0.05$).

Conclusions: Serum $Ca^{2+}$ levels significantly correlated with dengue severity. Serum $Ca^{2+}$ levels were significantly lower and hypocalcemia was more prevalent in patients with DHF than in patients with DF. Further studies are required to determine whether hypocalcemia can be utilized as a prognostic indicator and to evaluate effectiveness of calcium therapy in prevention of dengue complications.

**Key words:** dengue; dengue hemorrhagic fever; serum calcium.

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## Introduction

Dengue is a disease spread by the *Aedes* mosquito, and it is an entity known to mankind since 1780 [1]. After 1960, the incidence of dengue has shown an exponential increase, with several recent outbreaks reported mainly from South Asian countries [2]. Nearly 70% of the world's population at risk of dengue lives in the Southeast Asian and Western Pacific regions [2]. Dengue infection and dengue hemorrhagic fever (DHF) are major causes of morbidity and mortality in the tropical regions of the world [3]. It is estimated that 390 million become infected with dengue per year, of which 96 million manifest apparently [4]. Due to this high prevalence and considerable mortality, over the last few years there has been a heightened interest in disease prevention and effective strategies for management. However, at present, the pathogenesis of dengue and its complications are not completely understood. The dengue virus is a single-stranded RNA virus of the genus *Flavivirus*, comprising four distinct serotypes (DEN-1 to DEN-4) [5]. Currently, the most accepted theory is that of an abnormal or amplified immunological response occurring in a secondary infection with a different serotype than in the primary infection [6]. This results in an antibody-dependent enhancement of immunological reaction, resulting in endothelial injury, plasma leakage, reduced intravascular volume, and circulatory collapse [7]. Although no specific pathway has been identified linking known immunopathogenic events with definitive effects on microvascular permeability, thromboregulatory mechanisms, or both, preliminary data suggest that transient disruption in the function of the endothelial glycocalyx layer occurs, which probably enhances leakage [8].

Serum calcium is known to be important in cardiac and circulatory function. The administration of intravenous calcium has been a routine practice in resuscitation protocols for traumatic, hemorrhagic and

cardiogenic shock, a practice supported by the presence of hypocalcemia and the observed beneficial effects of calcium therapy in these conditions [9]. Known cardiovascular manifestations of hypocalcemia include hypotension, reduced myocardial function, electrocardiogram (ECG) abnormalities, and heart failure [10]. Alterations in calcium homeostasis, therefore, might play a role in the pathogenesis of shock in patients with dengue infection. Researchers have postulated that autonomic dysfunction might also contribute to hypotension in dengue shock syndrome (DSS) [11]. Calcium entry via neuronal calcium channels is essential for neurotransmission, hence calcium plays an important role in the smooth functioning of the autonomic nervous system [12]. Uddin *et al.* reported that the mean total calcium levels were significantly lower in patients with DHF than in patients with uncomplicated dengue fever (DF) [13]. However, free calcium is a more useful index than total calcium and provides a better indication of calcium status [14]. Calcium is transported predominantly bound to serum albumin; the total calcium level, therefore, is influenced directly by the serum albumin concentration. Numerous studies have clearly demonstrated that the measurement of free calcium is the test of choice in nearly all diagnostic and treatment situations [14]. In the present study, we evaluated the association between serum free calcium level and disease severity in patients with dengue infection. To our knowledge, this is the first study evaluating the association between the severity of dengue infection and serum free calcium levels.

## Methodology

*Study population and sampling*

A cross-sectional study was performed at a tertiary care private hospital in Colombo, Sri Lanka, for a period of six months in 2013. A consecutive sample of inpatients with confirmed dengue infection was recruited for the study, after written consent was obtained. The admission register at the hospital was used as the sampling frame. Patients with hypertension, diabetes and cardiac diseases and those on anti-hypertensive/anti-arrhythmic medications, calcium supplements, or any other drugs affecting calcium homeostasis were excluded, as these would alter the blood pressure, serum calcium levels, and ECG findings. Ethical approval for the study was obtained from the Ethics Review Committee, Faculty of Medicine, University of Colombo, Sri Lanka.

*Definitions*

A probable case of dengue was diagnosed according to the World Health Organization (WHO) criteria [15]. Confirmation of diagnosis was done with one of the following laboratory tests: IgM antibody (MAC-ELISA) (PANBIO diagnostics, Brisbane, Australia), dengue virus RT-PCR (single tube multiplex RT-PCR was carried out according to the standard method described previously [16]), or serum dengue NS1 (non-structural protein 1) antigen detection (PLATELIA TM Dengue NS1 Ag assay [BIORAD, Marnes-la-coquette, France]). DHF was diagnosed and classified in to four stages (DHF I-IV) according to the WHO criteria as follows: DHF I – positive tourniquet test and/or easy bruising; DHF II – presence of spontaneous bleeding manifestations; DHF III – circulatory failure (rapid, weak pulse and narrow pulse pressure or hypotension); and DHF IV – profound shock with undetectable pulse and blood pressure. The prevalence of myocarditis and its correlation with dengue severity was also analyzed. Myocarditis was diagnosed either by the presence of changes in the 12-lead ECG (ST segment, T inversion or right bundle branch block) or by the two-dimensional echocardiogram (2D-echo) findings (hypo-kinetic segments).

*Data collection and analysis*

Socio-demographic details were collected using an interviewer-administered structured questionnaire. The clinical parameters recorded were presence of suggestive symptoms (fever, headache, retro-orbital pain, arthralgia, myalgia, rash, and bleeding manifestations), evidence of fluid leakage (pleural effusion and ascitis), pulse rate, and systolic and diastolic blood pressure. In addition, the following investigations were performed: white cell count, platelet count, packed cell volume, serum free calcium level, ECG, and 2D echo. Blood samples for the estimation of serum calcium were drawn between days 5 and 10 of the fever. Hypocalcemia was defined as the presence of a serum free calcium level of < 1.1 mmol/L. All data were double-entered and cross-checked for consistency. Data were analyzed using SPSS version 14 (SPSS Inc., Chicago, IL, USA) statistical software package. The significance of the differences between proportions (%) and means were tested using the z-test and student's *t*-test or ANOVA, respectively.

## Results

The sample size was 135, and the mean age was 26.1 years (range 6–65 years). The majority of the patients were males (n = 80, 59.3%), and only 4 patients (3%) had a previous history of laboratory-confirmed dengue infection. The diagnosis was confirmed by using the dengue NS1 antigen, PCR, or IgM in 65 (48.1%), 1 (7.4%), and 39 (28.9%) patients, respectively. DHF was diagnosed in 71 patients (52.6%), of which 3 (4%) had DHF I, 34 (47.8%) had DHF II, and 29 (40.8%) had DHF III. There were no patients with DHF IV in the present cohort, and all patients recovered completely.

Complete data on serum free calcium was available only in 107 patients. The mean serum free calcium level of the study population was 1.05 mmol/L (range 0.77–1.24). The mean serum free calcium was significantly higher in patients with DF (1.09 mmol/L) than in those with DHF (1.02 mmol/L) ($p < 0.05$). The mean serum free calcium levels in the different stages of DHF were: DHF I – 1.076 mmol/L; DHF II – 1.022 mmol/L; and DHF III – 1.033. A significant difference was observed between DHF I and DHF II. Prevalence of hypocalcemia in DHF patients was 86.9% (n = 60), whereas it was 29.7% (n = 11) in patients with DF ($p < 0.05$).

Two-dimensional echo findings were available for 37 patients; no abnormalities were detected in 27 patients (72.9%). Features of myocarditis were present in 21.6% (n = 8) of patients, all of whom were in the DHF group. However, only 4 out of the above 8 patients had an ejection fraction of less than 60%. Dys-synchronic movements in ventricles were observed in 1 patient. ECGs were available in 51 patients, of which 80.4% (n = 41) had no abnormality. The commonest abnormality noted was T inversion in right or/and left leads, which was present in 9 (17.6%) patients. A right bundle branch block was present in 1 patient. QT changes and ST segment changes were not observed in the study population. Evidence of myocarditis (ECG and/or 2D echo) was seen in 16 patients, of which 14 (87.5%) were in the DHF group and 2 were in the DF group (12.5%) ($p < 0.05$).

## Discussion

Dengue is the most prevalent mosquito-borne viral infection in the world [17]. Each year, there are ~50 million dengue infections and ~500,000 individuals are hospitalized with DHF, mainly in Southeast Asia, the Pacific, and the Americas [18]. Sri Lanka is a middle-income developing country in the South Asian region with a population of over 20 million. It is an island nation with monsoon periods throughout the year and is thus a hot spot for dengue infections. The occurrence of dengue outbreaks in Sri Lanka dates back to the early 1900s, and since 2000, Sri Lanka has been struck by periodic outbreaks of dengue with a steady increase in the case fatality rate [19]. At present, novel and effective therapeutic and preventive strategies are of utmost importance.

To our knowledge, this is the first study evaluating the association between the severity of dengue infection and serum free calcium levels. Our results demonstrate that the serum free calcium levels significantly correlated with the severity of dengue. The mean serum free calcium was significantly lower in patients with DHF than in those with DF, and the prevalence of hypocalcemia was higher in patients with DHF than in patients with DF. A vast majority of deaths in dengue infections occur due to severe plasma leakage that occurs in DHF/DSS [17]. Therefore, the association between hypocalcemia and the severity of dengue needs to be further evaluated. The measurement of serum calcium is currently not a routine practice in patients with dengue infection. Further studies are required to determine whether the presence of hypocalcemia at the onset of the illness can be utilized as a prognostic indicator to predict disease severity. A pilot study conducted in Mexico on a limited number of patients with dengue infection demonstrated that oral $CaCO_3$ plus vitamin $D_3$ supplementation improved the overall clinical condition and reduced the duration of illness [20]. In a similar study, oral $CaCO_3$ supplementation significantly increased the number of platelets in patients with dengue infection when compared with a control group [21]. However, there are currently no randomized control trials evaluating the effectiveness of calcium therapy in the prevention of complications in dengue infection. Hence, oral or IV calcium therapy is not routinely included in published guidelines. Furthermore, hypocalcemia has also been demonstrated in certain cases of malaria, severe meningococcal infections, and other severe acute illnesses, being associated with a poor prognosis [22-24].

Previously, we postulated that transient sympathetic blunting or failure could be a mechanism partially responsible for blood pressure changes in DHF [11]. However, serum calcium level changes have to be analyzed further to see whether they play a role in transient sympathetic blunting or failure. Further human and animal studies are required with special reference to the place of calcium in

sympathetic dysfunction. Furthermore, the exact mechanism for hypocalcemia in severe dengue infections also requires further study. Possible mechanisms include leak into the potential third spaces, disturbance in cellular transport, or changes in hormones involved in calcium metabolism. There are several limitations to our study. Our results are from a cross-sectional analysis; however, prospective cohort studies are required to determine whether low serum free calcium is a risk factor for the development of complications in patients with dengue. In addition, dengue infection was diagnosed using different tests (NS1 antigen, PCR, and IgM), with varying sensitivities, specificities, and limitations [25]. Furthermore, blood samples for estimation of serum calcium were taken on different days, and from different patients, which could have influenced the results. However, it was necessitated by the different times of presentation of patients and by the commercial availability.

## Conclusions

We demonstrate that the serum free calcium levels significantly correlated with the severity of DF. The serum free calcium levels were significantly lower and hypocalcemia was more prevalent in patients with DHF than in those with DF. Further studies are required to determine whether the presence of hypocalcemia can be utilized as a prognostic indicator in dengue infection. In addition, randomized control trials are required to evaluate the effectiveness of calcium therapy in the prevention of complications in dengue infection.

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