

## Association of Hypocalcemia with Disease Severity in Patients of Dengue - An Observational Study

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### Abstract

**Background:** Dengue is a vector-borne disease known to mankind since 1780. The main culprit being the dengue virus which is transmitted by Aedes mosquito. Dengue infection and Dengue hemorrhagic fever (DHF) are the major causes of morbidity and mortality in the tropical regions all over the world. An estimate reveals that 390 million people become infected with dengue every year, of which 96 million manifests apparently. Dengue virus is an arbovirus which belongs to the genus Flaviviridae.

**Aim and Objectives:** To analyze the association of hypocalcemia with disease severity in patients of dengue as well as the prevalence of hypocalcemia and its potential value as a biochemical marker in patients with severe dengue infection.

**Material and Methods:** This was an Observational cross-sectional descriptive study started from June 2019 onwards for One year, conducted at Department of General Medicine, SMS Medical College and attached group of hospitals, Jaipur, Rajasthan. After application of inclusion and exclusion criteria sample size was calculated 90 cases of Dengue as per previous studies, showing prevalence of hypocalcemia 29.9 % with 80% power,  $\alpha=0.05$  error and 10% absolute error.

**Result:** In this study with the Sample size of 90 patients we found that 67.8% patients were less than or equal to 30 years of age, followed by 23.3% patients were between 31-45 years. Out of 90 patients 64.4% were having more than or equal to 4.7 mg/dL Ionic calcium and 35.5% patients were having less than 4.7 mg/dL Ionic calcium. 60 patients suffered from uncomplicated dengue fever out of which 53 patients were having ionic calcium more than 4.7mg/dl and 7 patients were having less than 4.7mg/dl ionic calcium whereas 30 patients suffered from Severe complicated dengue including DSS and DHF out of which 5 patients had ionic calcium more than 4.7mg/dl and 25 patients had less than 4.7mg/dl ionic calcium.

**Conclusion:** From this study we demonstrate that the serum free calcium levels significantly correlated with the severity of Dengue fever. The serum free calcium levels were significantly lower and hypocalcemia was more prevalent in patients with DHF/DSS than in those with DF. Further studies and randomized control trials are required to evaluate the effectiveness of calcium therapy in the prevention of complications in dengue infection.

**Keywords:** Hypocalcemia, Severity, Dengue.

**Abbreviations:** DF - Dengue Fever , DHF - Dengue Hemorrhagic Fever, DSS - Dengue Shock Syndrome

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**Introduction**

Dengue is a vector-borne disease caused by dengue virus and is transmitted by *Aedes* mosquito, and it is a disease known to mankind since 1780. [1] The origin of word —denguel is derived from the Swahili phrase “kadinga pepo” which describes the disease is being caused by an evil spirit. There is 30-fold increase in global incidence of dengue over the last five decades. [2] Dengue infection and dengue hemorrhagic fever (DHF) being the major causes of morbidity and mortality in the tropical regions all over the world. [3] It is estimated that 390 million people become infected with dengue every year, of which 96 million manifests apparently. [4] In India, every year cases are spreading to newer geographical areas. [2] The disease has a seasonal pattern; the cases peak after the monsoons and are not uniformly distributed throughout the year. [5] The exact pathogenetic mechanism for different clinical manifestations of dengue fever is still not clearly understood. Various mechanisms are proposed to explain signs and symptoms such as complex immune mechanism, T-cell mediated antibodies cross reactivity with vascular endothelium, enhancing antibodies, complement and its products and various soluble mediators including cytokines and chemokines.

Dengue virus is arthropod borne RNA virus which belongs to the genus *Flaviviridae*. Four serologically distinct dengue virus have been identified till now DENV-1, DENV-2, DENV-3 and DENV-4. According to WHO 2009 classification, severe dengue infection (SDI) is defined by one or more of the following:

- (i) Plasma leakage that may lead to shock (dengue shock) and/or fluid accumulation, with or without respiratory distress, and/or
- (ii) Severe bleeding, and/or
- (iii) Severe organ impairment, of which most occur during the critical phase. [6]

The calcium ion plays a major role in normal functioning of cells, regulating the physiological processes such as neuromuscular transmission, cardiac contractility, hormone secretion and blood coagulation. The total calcium level depends directly on the serum albumin level. Therefore, the free calcium is more useful than total calcium in providing information about calcium status of the body. [7] This study aimed to analyze the association of hypocalcemia with disease severity in patients with dengue as well as the prevalence of hypocalcemia and its potential value as a biochemical marker in patients with severe dengue infection.

**Materials and methods**

This was an Observational cross-sectional descriptive study started from June 2019 onwards for One year, conducted at Department of General Medicine, SMS Medical College and attached group of hospitals, Jaipur, Rajasthan. After application of inclusion and exclusion criteria; as per previous studies sample size was found to be 90 cases of Dengue showing prevalence of hypocalcemia 29.9 % with 80% power,  $\alpha$  0.05 error and 10% absolute error.

Serum ionic calcium level of the patients was measured within first 24 hour of admission. Ionic calcium calculated by formula  $iCa = [0.9 + (0.55 \times t Ca - 0.3 \times$

albumin)] .The resulting study group was categorized into normal ionised serum calcium level and decreased ionised calcium level and the relevant data was analyzed with appropriate statistical tools.

**Inclusion criteria:-**

1. Dengue IgM and NS1 antigen positive patients
2. Patients >18 years age
3. Patients willing to give written informed consent
4. Patients without previous history of dengue infection

**Exclusion criteria:-**

1. Patients with other causes of hypocalcemia i.e malaria, meningococemia and leptospirosis were excluded

2. Patients on calcium supplements, other drugs affecting calcium homeostasis, PTH deficiency, renal disorder.

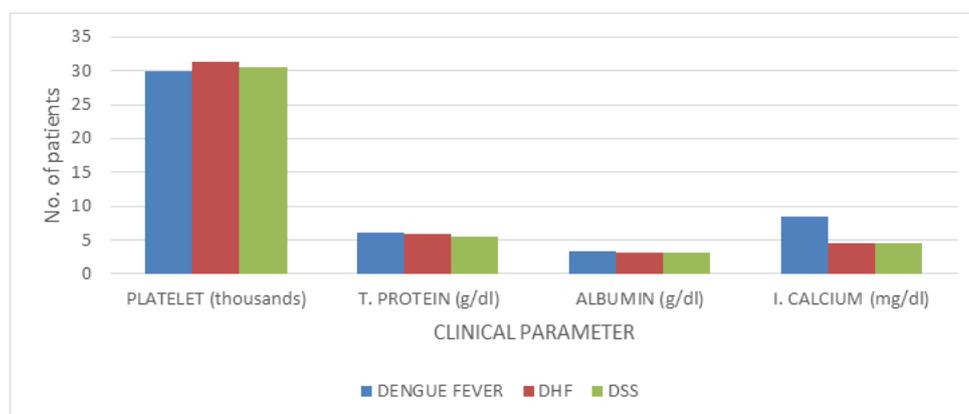
**Results**

In this study with sample size of 90 we found that 67.8% patients were less than or equal to 30 years, followed by 23.3% patients were between 31-45 years, 3.33% patients were between 45-60 years and 5.6% patients were more than 60 years. Mean age was 28.88 years in the study group. Out of total cases 58.88% were male and 41.11% were female. According to the study, 72.22% patients categorised under only IgM positive, 18.88% patients categorised under only NS1Ag positive and 8.88% patients included under both IgM+ NS1Ag antigen positive.

**Table No:1 Distribution According to Ionic Calcium**

IONIC CALCIUM (mg/dL)	NO. OF PATIENTS	PERCENTAGE
<4.7	32	35.5
≥4.7	58	64.4
TOTAL	90	100

In this study we observed that, 64.4% patients were having more than or equal to 4.7 mg/dL Ionic calcium and 35.5% patients were having less than 4.7 mg/dL Ionic calcium. 80% patients were having no Comorbidity, 6.66% patients were having HTN, 5.55% patients were of T2DM, 5.55% patients were having COPD and 3.33% patients were known cases of T1DM.



**Figure 1: Comparison between Clinical Parameter and Severity**

Out of total 90 patients, 66.66% patients were of uncomplicated dengue fever,

31.11% patients were of DHF and 2.22% patients were of DSS. According to

collected data mean and standard deviation were calculated for various biochemical parameters. The mean total calcium was 7.6 mg/dl and SD was 0.85. The mean  $\pm$  SD of SGOT was 446.75 U/L  $\pm$  119.54,

mean  $\pm$  SD of SGPT was 400.83 U/L  $\pm$  118.8 , mean  $\pm$  SD of PLATELET was 31.52  $\pm$  20.75 and the mean  $\pm$  SD ionic calcium ( iCa ) was 5.1 mg/dl  $\pm$  0.79 accordingly.

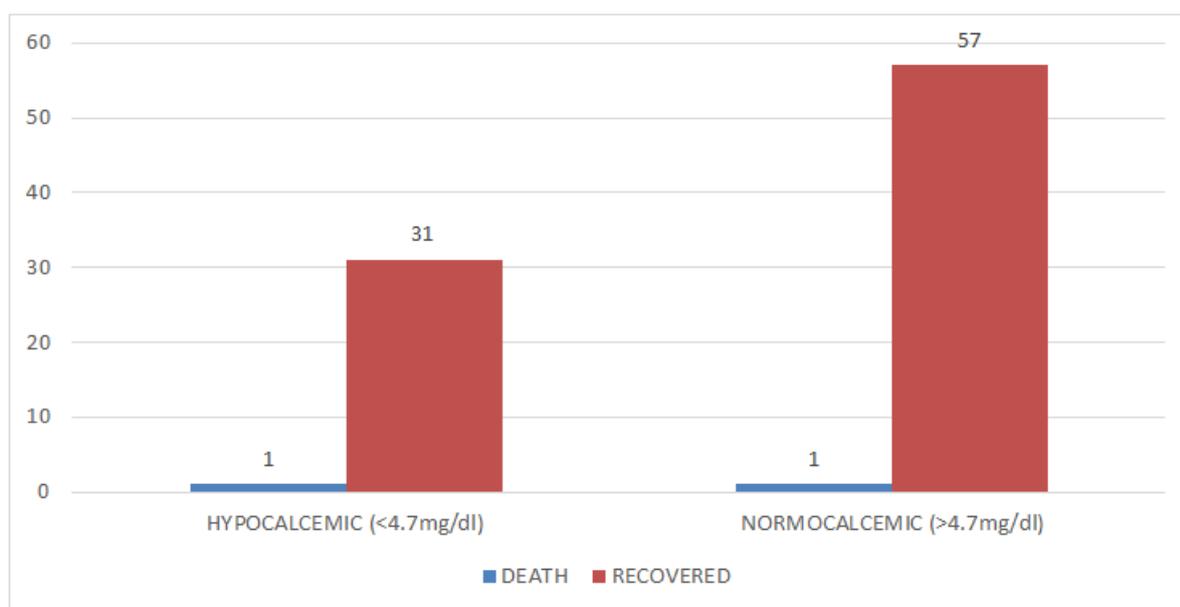
**Table No:3 Comparison between Biochemical Parameter and Severity**

BIOCHEMICAL PARAMETER	DENGUE FEVER	DHF	DSS	P VALUE
IPF	14.33 $\pm$ 7.79	15.9 $\pm$ 7.32	11.4 $\pm$ 4.5	0.54
INR(seconds)	1.08 $\pm$ 0.52	1.016 $\pm$ 0.21	2.6 $\pm$ 1.8	0.0001
SGOT(U/L)	444.9 $\pm$ 120.3	461.07 $\pm$ 124.4	644.5 $\pm$ 204.3	0.07
SGPT(U/L)	403.5 $\pm$ 122.2	408.46 $\pm$ 118.66	555 $\pm$ 281.4	0.24

In this study, 60 patients suffered from uncomplicated dengue fever out of which 53 patients were having ionic calcium more than 4.7mg/dl and 7 patients were having less than 4.7mg/dl ionic calcium whereas 30 patients suffered from Severe complicated dengue including DSS and

DHF out of which 5 patients had ionic calcium more than 4.7mg/dl and 25 patients had less than 4.7mg/dl ionic calcium.

The p value was calculated by ANOVA test which was statistically significant (p <0.05)



**Figure 2: Distribution According to severity of hypocalcemia and Outcome**

According to the study, out of total 32 patients of dengue with hypocalcemia, 96.8% patients recovered and 3.1% died. we also observed that out of total 58 patients, who had normal serum ionic calcium levels, 98.33% patients recovered while 1.66% patients died. According to statistical analysis we found that there is

no significant relation between mortality rate and hypocalcemia in dengue patients.

**Discussion**

Dengue is the most prevalent mosquito-borne viral infection in the world. [8] 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. [9] Dengue infection is potentially a fatal illness. Fluid management, support of the involved organs and correction of metabolic derangement is the mainstay of treatment. Calcium plays a vital role in the platelet aggregation and in the functioning of myocardial tissue. [10] Calcium homeostasis derangements are probably associated with cardiac arrhythmias and myocardial dysfunction. [11] Calcium homeostasis derangements are observed in dengue as documented by in vitro studies. [10]

In this study we observed that, 64.4% patients were having more than or equal to 4.7 mg/dL Ionic calcium and 35.5% patients were having less than 4.7 mg/dL Ionic calcium. Out of total 90 patients, 66.66% patients were of uncomplicated dengue fever, 31.11% patients were of DHF and 2.22% patients were of DSS.

According to the study, 60 patients suffered from uncomplicated dengue fever out of which 53 patients were having ionic calcium more than 4.7mg/dl and 7 patients were having less than 4.7mg/dl ionic calcium whereas 30 patients suffered from Severe complicated dengue including DSS and DHF out of which 5 patients had ionic calcium more than 4.7mg/dl and 25 patients had less than 4.7mg/dl ionic calcium. The p value was calculated by ANOVA test which was statistically significant ( $p < 0.05$ ). Comparable to this study Uddin et al [12], noted significantly lower mean total calcium levels in DHF/DSS patients than with uncomplicated dengue fever patients.

Total protein, Albumin and platelets showed no statistically significant difference ( $p$ -value  $> 0.05$ ). Their mean and standard deviation are mentioned in Table 2 above.

In this study, Mean and standard deviation were calculated for various biochemical

parameters as mentioned above in Table no. 3.  $p$ -value for IPF, SGOT, SGPT was 0.54, 0.07 and 0.24 respectively. These parameters showed non-significant difference as  $p$ -value was  $> 0.05$ . While comparing mean  $\pm$  SD of various biochemical parameters with severity of dengue, we found that Ionic calcium showed statistically significant difference as its  $p$ -value was  $< 0.05$ . Similarly, Constantine et al [13], also showed that mean ionized calcium levels were lower in DHF (1.02 mmol/l than in those with DF [1.09])  $p < 0.05$ .

According to the study, out of total 32 patients of dengue with hypocalcemia, 96.8% patients recovered and 3.1% died. we also observed that out of total 58 patients, who had normal serum ionic calcium levels, 98.33% patients recovered while 1.66% patients died. According to statistical analysis we found that there is no significant relation between mortality rate and hypocalcemia in dengue patients. Suresh S R et al [14] found that out of 9 patients with severe dengue, 3 patients succumbed to death. The mortality rate in their study was 3%. This result is according to our study.

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 uncomplicated 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. [10] Therefore, the association between hypocalcemia and the severity of dengue needs to be further evaluated.

A pilot study conducted in Mexico on a limited number of patients with dengue infection demonstrated that oral  $\text{CaCO}_3$  plus vitamin D3 supplementation improved the overall clinical condition and reduced the duration of illness. [15]

In a similar study, oral CaCO<sub>3</sub> supplementation significantly increased the number of platelets in patients with dengue infection when compared with a control group. However, there are currently no randomized control trials evaluating the effectiveness of calcium therapy in the prevention of complications in dengue infection. [16,17]

### Conclusion:

From this study we demonstrate that the serum free calcium levels significantly correlated with the severity of Dengue fever. The serum free calcium levels were significantly lower and hypocalcemia was more prevalent in patients with DHF/DSS 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.

### Reference

- Godwin R Constantine et.al, Hypocalcaemia is associated with disease severity in patients with dengue. *J Infect Dev Ctries* 2014; 8(9):1205- 1209.
- Ashutosh Biswas et.al, Indian National Guidelines for Clinical Management of Dengue Fever. *J of the Ind Med Assoc*, Vol. 113, No. 12, December 2015.
- Gubler DJ (1997) The emergence of dengue/dengue hemorrhagic fever as a global public health problem. In: Saluzzo JF, Dodet B, editors. *Factors in the emergence of arbovirus diseases*. Paris: Elsevier. 83-92.
- Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, Myers MF, George DB, Jaenisch T, Wint GRW, Simmons CP, Scott TW, Farrar JJ, Hay SI (2013) The global distribution and burden of dengue. *Nature* 496: 504-507.
- Adikari M, Perera C, Fernando M, Loeb M Premawansa S, et al. (2015) Prevalence of Hypocalcaemia and Its Potential Value as a Biochemical Marker in Patients with Severe Dengue Infection. *J Trop Dis* 4: 188.
- World Health Organization (2009) *Dengue guidelines for diagnosis, prevention and control*, New edition. Geneva.
- Sava L, Pillai S, More U, et al. Serum calcium measurement: total versus free (ionized) calcium. *Indian J Clin Biochem* 2005;20(2):158-61.
- Guzman MG, Kouri G (2002) Dengue: an update. *Lancet Infect Dis* 2: 33-42.
- Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, et al. (2010) Dengue: a continuing global threat. *Nat Rev Microbiol* 8(12 Suppl): S7-16.
- hivanthan MC, Rajapakse S. Dengue and calcium. *Int J Crit Illness Injury Sci*. 2014;4(4):314.
- Dimopoulou I. Endocrine and metabolic disturbances in critically ill patients: to intervene or not? *Eur J Intern Med*. 2005; 16:67-68
- Uddin KN, Musa AKM, Wasim M, Haque M, Sarker RSC, et al. (2008) A follow up on biochemical parameters in dengue patients attending Birdem hospital. *Ibrahim Med Coll J* 2: 25-27.
- Godwin R Constantine et.al, Hypocalcaemia is associated with disease severity in patients with dengue. *J Infect Dev Ctries* 2014; 8(9):1205-1209.
- Suresh SR, Srikanth AK, K. Sriharsha, et al. Utility of serum free calcium as a predictor of severity in dengue fever. *J. Evolution Med. Dent. Sci*. 2018;7(39):4277-4281.
- Sanchez-Valdez E, Delgado-Aradillas M, Torres-Martinez JA, Torres-Benitez JM (2009) Clinical response in patients with dengue fever to oral calcium plus vitamin D administration: study of 5 cases. *P W Pharmacol Soc* 52: 14- 17.

16. Berthelot, M., Rieker, A., & Correia, J. C. The difficulties experienced by patients with low back pain in France: a mixed methods study. *Journal of Medical Research and Health Sciences*, 2022;5(6), 2039–2048.
17. Cabrera-Cortina JI, Sanchez-Valdez E, Cedas-DeLezama D, Ramirez-Gonzalez MD. Oral calcium administration attenuates thrombocytopenia in patients with dengue fever. Report of a pilot study. *P W Pharmacol Soc* 2008;51: 38-41.