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A cross sectional study on the relationship between serum calcium level and severity of dengue fever

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***Abstract*---**Background: Dengue is the most rapidly spreading mosquito borne disease in the world. An estimated 50 million dengue infections occur annually. It is caused by a small, single stranded RNA virus belonging to the genus Flavivirus, family Flaviviridae and is divided into four serotypes (DEN-1 to DEN-4). These various serotypes are transmitted to humans through the bites of infected Aedes mosquitoes, primarily Aedes aegypti. Materials & Methods: A cross sectional study was conducted on 72 patients with dengue fever, for a period of 12 months in the medicine wards of Government Medical College, Kottayam. All cases fulfilling the inclusion criteria during the study period were included after SRC and IRB approval. Results: Mean age of patients in the present study was 32.6 in dengue fever and 32.1 in severe dengue. There was no significant correlation between age and serum corrected calcium level (Pearson correlation coefficient – 0.074, p value 0.539). In patients with dengue fever, mean hematocrit was 39.2 %, while in those with severe dengue, it was 40.5 %. There was no significant correlation between hematocrit and serum corrected calcium level (Pearson correlation coefficient – 0.147, p value 0.219). The area under the receiver operating characteristic (ROC) curve was 0.962, indicating a high accuracy for serum corrected calcium level in detecting severe dengue. Conclusion: Serum corrected calcium levels were found to be significantly lower in severe dengue when compared to dengue fever. Serum corrected calcium levels correlate with severity of dengue fever and can be used as a prognostic marker for dengue fever.

***Keywords*---**Serum calcium level, Severity of dengue fever, A cross sectional study.

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Introduction

Dengue is the most rapidly spreading mosquito borne disease in the world. An estimated 50 million dengue infections occur annually. It is caused by a small, single stranded RNA virus belonging to the genus Flavivirus, family Flaviviridae and is divided into four serotypes (DEN-1 to DEN-4).[1] These various serotypes are transmitted to humans through the bites of infected Aedes mosquitoes, primarily Aedes aegypti. As per the Dengue guidelines given by WHO in 2009, Dengue fever has been classified into: Dengue fever with no warning signs, Dengue fever with warning signs and Severe dengue.[2] Although the WHO severity classification of dengue is used here, certain studies show that only WHO criteria of severity may not be sufficient to categorize and treat the patients of dengue, especially those with complications. [3,4]

Calcium ion plays an important role in normal cell signaling and cellular function. Serum calcium level is divided into three fractions:

I. Ionized (free) calcium – 50 % of the total
II. Protein bound (albumin) – 40 % of the total
III. Anion bound (lactate, phosphate) – 10 % of the total

Hypocalcemia is defined as a serum corrected calcium level less than 8.5 mg/dL. Hypocalcemia has been described in dengue fever and is more marked in severe dengue – the precise mechanisms of which are not clearly defined. The probable causes for hypocalcemia that have been proposed include – reduced Na^+-K^+ ATPase activity, acquired parathyroid hormone deficiency, renal one-alpha hydroxylase insufficiency, reduced dietary vitamin D and calcium intake. [5]

Various studies have shown serum calcium to be significantly lower in patients with severe dengue, hence serum corrected calcium level could be used as an important biomarker to assess severity of dengue fever and plan management accordingly. This study aims to correlate the severity of dengue fever with the level of serum corrected calcium.

Objectives

To estimate the relationship between serum, mean corrected calcium level and the severity of dengue fever in patients admitted in medicine wards in Government Medical College, Kottayam.

Materials & Methods

After obtaining due ethical clearance from the Ethical Committee of Government Medical College, Kottayam and Department of General Medicine, a cross sectional study was conducted on 72 patients with dengue fever, for a period of 12 months in the medicine wards of Government Medical College, Kottayam. All cases fulfilling the inclusion criteria during the study period were included after SRC and IRB approval. Informed consent was obtained from the patients.

Sample Size

Using the values of mean and standard deviation from the study done in BIRDEM Hospital, Dhaka by Uddin et al. with 95% confidence interval and 95% power, the minimum sample size calculated was 72 dengue fever patients. [6]
Sample size = 72

Inclusion criteria
All patients admitted in Medicine wards with serologically confirmed Dengue fever (Dengue NS1/ IgM positive) in the age group 18-60 years.

Exclusion Criteria
Patients on calcium supplements or drugs affecting calcium homeostasis.

Study Procedure
A detailed structured proforma was completed and according to the history, examination findings and laboratory investigations, patients were classified into 2 groups: Dengue fever (with / without warning signs) and severe dengue. Serum corrected calcium level was assessed in these patients within 24 hours of admission using electrochemistry (calcium sensing electrode) and then correlated with the group of dengue fever the patient falls into.

Data management and statistical analysis

Data was coded and entered in Microsoft Excel and analyzed using IBM SPSS software Version 22. The significance of the difference between means of the two groups was tested using student's t-test. A 'p' value less than 0.05 was considered significant. Charts and tables were generated with the help of Microsoft Excel.

Results

A total of 72 patients were dengue fever were included in this study. The diagnosis of dengue fever was confirmed with Dengue NS1 antigen or Dengue IgM ELISA.

Table 1 Age & gender distribution of dengue patients

Age group distribution of dengue patients		
Age in years	Frequency	Percentage
≤20	12	16.7
21 – 30	28	38.9
31 – 40	15	20.8
41 – 50	10	13.9
>50	7	9.7
Total	72	100
Gender distribution of dengue patients		
Sex	Frequency	Percentage
Male	51	70.8
Female	21	29.2
Total	72	100

The mean age of the study participants was 30 years. The minimum age was 18 years and maximum age was 60 years. Majority of the study population, 28 (38.9 %) were in the age group of 21-30 years. 51 (70.8 %) of the study population were males.

Table 2 showing the age, serum corrected calcium, Hematocrit, platelet count (on admission) & SGPT vs severity of dengue fever

Age vs severity of dengue fever					
		Age (in years)			
Dengue Fever	N	Mean	S.D	t	P
Dengue fever	39	32.6	11		
Severe dengue fever	33	32.1	14	0.167	0.868
Serum corrected calcium vs severity of dengue fever					
Dengue Fever	N	Serum Corrected Calcium (mg/dL)		t	P
		Mean	S.D		
Dengue fever	39	8.9	0.4		
Severe dengue fever	33	8.2	0.2	9.305	<0.001
Hematocrit vs severity of dengue fever					
		Hematocrit			
Dengue Fever	N	Mean	S.D	t	P
Dengue fever	39	39.7	2.7		
Severe dengue fever	33	40.5	5.9	0.839	0.404
Platelet count (on admission) vs severity of dengue fever					
		Platelet count on admission (/mm^3)			
Dengue Fever	N	Mean	S.D	t	P
Dengue fever	39	81923.1	36434.1		
Severe dengue fever	33	39606.1	34734.7	5.016	<0.001
SGPT vs severity of dengue fever					
Dengue Fever	N	SGPT (IU/L)		t	P
		Mean	S.D		
Yes	39	89	59.2	4.894	<0.001
No	33	460.5	470.3		

39 patients of the 72 (54.2 %) belonged to the dengue fever, while 33 (45.8 %) were classified as having severe dengue. Of the severe dengue patients, 4 patients (12 %) developed severe transaminitis (SGPT > 1000 IU/L) and 2 patients (6 %) died during the course of the illness.

Serum corrected calcium level was measured during the first 24 hours of admission. The mean corrected calcium level in severe dengue patients (8.2 +/- 0.4 mg/dL) was significantly lower than that of dengue patients (8.9 +/- 0.2 mg/dL) (p value < 0.001)

There was a statistically significant association between serum corrected calcium level and severity of dengue fever. There was no significant association between age and severity of dengue fever (p velue 0.868) or between hematocrit and severity of dengue fever (p value 0.404). There was a positive correlation (Pearson correlation coefficient + 0.655) between platelet count on admission and serum corrected calcium level (p < 0.001). There was a negative correlation (Pearson correlation coefficient - 0.57) between SGPT and serum corrected calcium level (p < 0.001).

Table 3 Correlation of Serum corrected calcium with individual variables

Correlation of Serum Corrected Calcium with other variables	Pearson Correlation coefficient	P
Age	-0.074	0.539
Hematocrit	-0.147	0.219
Platelet count on admission	0.655**	<0.001
SGPT	-0.570**	<0.001

There was no significant correlation between age and serum corrected calcium level (Pearson correlation coefficient – 0.074, p value 0.539). There was no significant correlation between hematocrit and serum corrected calcium level (Pearson correlation coefficient – 0.147, p value 0.219).

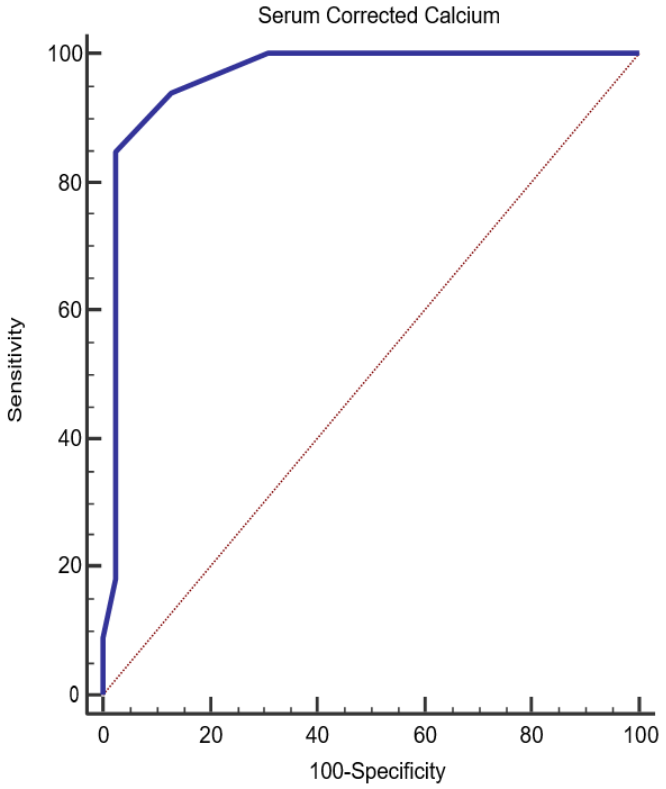

Figure 1 Receiver operating characteristic (ROC) curve of serum corrected calcium

The area under the receiver operating characteristic (ROC) curve was 0.962, indicating a high accuracy for serum corrected calcium level in detecting severe dengue.

Table 4 Analysis of ROC curve of serum corrected calcium

Dengue fever	
Sample size	72
Severe Dengue fever	33
Dengue fever	39
Area under the ROC curve (AUC)	0.962
Standard Error	0.024
95% Confidence interval	0.888 to 0.993
Youden index J	0.8228
Optimum cut off	≤8.4
Sensitivity	84.85
Specificity	97.44

The optimum cut off for serum corrected calcium was calculated as < 8.4 mg/dL with a sensitivity of 84.85, specificity of 97.44, positive likelihood ratio of 33.09,

negative likelihood ratio of 0.16, positive predictive value of 96.6, and negative predictive value of 88.4.

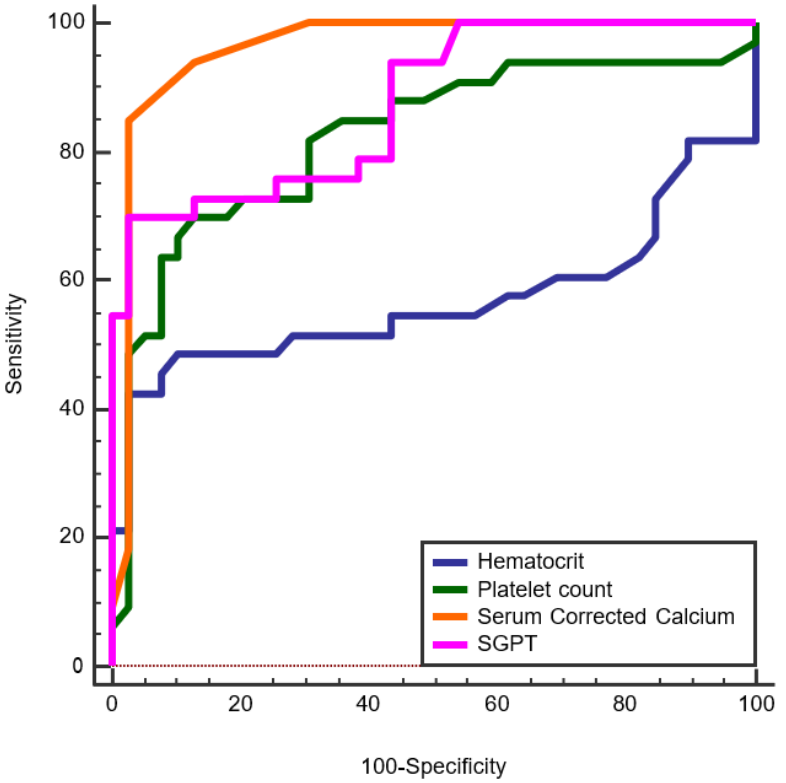

Figure 2 Receiver operating characteristic (ROC) curve of hematocrit, platelet count, serum corrected calcium and SGPT

The ROC curves of Platelet count (Area under the curve – 0.822) and SGPT (Area under the curve – 0.875) also showed high diagnostic accuracies in detecting severe dengue cases.

Discussion

In this study, a total of 72 patients were studied over a period of 12 months, of which 70.8 % were males and majority were in the age group of 21-30 years [38.9 %] and a mean age of 30 years [range 18-60 years]. A similar study was done by Uddin et al in BIRDEM hospital on 84 patients, of which 61.9 % were males, with age ranging between 26 to 63 years. [6] An observational prospective was study done in Amrita Institute of Medical Sciences, Kochi by M.R. Murugan, M. Gopalakrishna Pillai in 2015, on 68 patients, of which 60 % were males, with a mean age of 32 years [range 18 – 50 years]. [7]

Association between serum corrected calcium level and severity of dengue fever

In this study, serum corrected calcium level was found to be significantly lower in severe dengue (8.2 +/- 0.2 mg/dL) as compared to patients with dengue fever (8.9 +/- 0.4 mg/dL), indicating a relationship between serum calcium level and severity of dengue fever. The study conducted by Uddin et al in Ibrahim Medical College and BIRDEM Hospital, Dhaka, Bangladesh in 2002, on 84 dengue patients, showed a mean serum calcium level of 8.69 +/- 0.68 mg/dL in dengue fever, and lower (7.83 +/- 0.66 mg/dL) in dengue hemorrhagic fever– III.[6] The study reported that a significant number of patients with dengue fever had hypocalcemia (some were symptomatic) and concluded that mean serum calcium levels correlated with the severity of dengue fever.

An observational prospective study done in Amrita Institute of Medical Sciences, Kochi by M.R. Murugan, M. Gopalakrishna Pillai in 2015, on 68 patients with dengue fever showed that serum calcium levels were significantly lower in patients with severe dengue (8.48 +/- 0.43 mg/dL) when compared to dengue fever (7.83 +/- 0.42 mg/dL).[7] They concluded that serum calcium can be used as a marker of severity of dengue fever. Platelet counts on admission in this study also showed significantly lower levels in those with severe dengue [39,606] than in those with dengue fever [81,923].

In the study by Uddin et al, lower platelet counts were seen in severe dengue [24,750] (Dengue Hemorrhagic fever Class III as per old WHO classification) than in dengue hemorrhagic fever class I [43,719], suggesting that lower platelet counts were seen in severe dengue [6]. Similarly, in a study done by Aditya Mahajan et al. in A J Institute of Medical Sciences, platelet count in severe dengue [17,791] was significantly lower than that in dengue fever with warning signs [56,050] and in dengue fever without warning signs [1,18,166] [8].

Serum glutamic pyruvic transaminase (SGPT) or Alanine transaminase (ALT) in this study was found to be higher in severe dengue [460.5] when compared to dengue fever [89]. In the study by Uddin et al., SGPT was also found to be higher in severe dengue fever [582] (Dengue hemorrhagic fever class III as per old WHO classification) when compared to dengue fever [140]. [6]

Similarly, the study by Aditya Mahajan et al. also showed higher levels of SGPT in severe dengue [420], when compared with dengue fever with warning signs [129] and dengue fever without warning signs [43], suggesting that higher SGPT values were seen in severe dengue. [8]

Conclusion

Serum corrected calcium levels were found to be significantly lower in severe dengue when compared to dengue fever. Serum corrected calcium levels correlate with severity of dengue fever and can be used as a prognostic marker for dengue fever.

Limitations

The days on which serum calcium assays were done could not be standardized as patients presented on different days after symptom onset.

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