UTILITY OF SERUM FREE CALCIUM AS A PREDICTOR OF SEVERITY IN DENGUE FEVER

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BACKGROUND

Dengue fever causes considerable mortality and morbidity. The calcium ion plays a major role in cellular functions and signalling. We evaluated the correlation between severity of dengue and the serum free calcium.

MATERIALS AND METHODS

A cross-sectional study was done in a tertiary care hospital in Bapuji and Chigateri Hospital, Davangere. Patients with dengue fever were diagnosed using NS1 antigen and/or IgM antibody tests and were classified according to WHO criteria. The association between severity of dengue and serum free calcium was studied.

RESULTS

Sample size was 100. Among them, 68 (68%) were males and 32 (32%) were females. 37 (37%), 54 (54%) and 9 (9%) patients were classified as Dengue Fever (DF), Dengue Fever with Warning Signs (DF + WS) and Severe Dengue (SD) respectively. The mean serum free calcium in the study was $3.92 \pm 0.50 \text{ mg/dL}$. The mean serum calcium (mg/dL) in each class were as follows- Severe Dengue (3.09 ± 0.16), Dengue Fever with Warning Signs (3.72 ± 0.34) and Dengue Fever (4.41 ± 0.16). The presence of hypocalcaemia significantly correlated with the severity of dengue (p < 0.001).

CONCLUSION

We conclude that the serum free calcium levels significantly correlated with the severity of dengue fever. Serum free calcium can be used as a predictor of severity in dengue fever.

KEY WORDS

Dengue, Hypocalcaemia, Serum Free Calcium, Severe Dengue.

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BACKGROUND

Dengue is a mosquito-borne disease caused by dengue virus (DENV), which leads to considerable mortality and morbidity worldwide. It is an ancient disease. The first record of a case of probable dengue fever is in a Chinese medical encyclopaedia from Jin Dynasty (265 – 420 AD), which referred to a "water poison" associated with flying insects with symptoms of fever, rash, eye pain, joint pains and haemorrhage. The name dengue is purportedly derived from a Swahili phrase "Ka-dinga pepo," a disease of the devil. The first confirmed case report dates from 1789 and is by Benjamin Rush¹ who coined the term "breakbone fever."

Dengue infection is a major health problem in our country. The incidence of dengue fever is increasing in the recent years. The WHO estimates that about two-fifths of the world population is at risk for this viral infection. Every year during the monsoon months, many parts of the country will have outbreaks of dengue infection.²

Financial or Other Competing Interest': None. Submission 06-08-2018, Peer Review 08-09-2018, Acceptance 14-09-2018, Published 24-09-2018. Corresponding Author: Dr. Srikanth A. K, Postgraduate Student, Department of General Medicine, Jagadguru Jayadeva Murugarajendra Medical College, Davangere, Karnataka, India. E-mail: srikanth.a.k241@gmail.com DOI: 10.14260/jemds/2018/954 Dengue virus (DENV) is a single stranded RNA virus of family Flaviviridae, genus flavivirus. There are four types of dengue virus- DENV1, DENV2, DENV3, DENV4. The viral genome is translated to form a polypeptide which is then cut into 10 proteins; 3 structural proteins- Capsid (C), Membrane (M) and Envelope (E) and 7 Non-Structural (NS) proteins-NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5. The non-structural proteins are responsible for viral replication and assembly.

Halstead³ in 1970s proposed the "antibody-dependent immune enhancement theory" for pathogenesis of dengue. Studies have shown that secondary dengue infections showed predominant expansion of T cells with low avidity for the current infecting viral serotype and high avidity for a presumed previous serotype.⁴ This activation of memory CD4+ and CD8+ T cells, sensitised during a previous infection leads to rapid proliferation and release of pro-inflammatory cytokines, "cytokine tsunami," particularly TNF α and IFN γ .⁵ In severe dengue the low potentiality of T cells fails to control the viral replication, leading to excessive release proinflammatory cytokines causing tissue damage and plasma leakage.

Dengue can present with a wide-spectrum of clinical features, ranging from a simple fever to severe dengue in the form of plasma leakage leading to hypovolaemic shock. Dengue was previously classified into dengue fever and Dengue Haemorrhagic Fever (DHF), which has four grades with DHF III and IV compiling Dengue Shock Syndrome (DSS). In 2009, the WHO⁶ reclassified dengue because of difficulty in applying the old classification system in clinical situations.

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The WHO's 2009 classification divides dengue fever into two groups: Uncomplicated (Dengue Fever and Dengue Fever with Warning Signs) and Severe. Severe dengue is associated with bleeding manifestation, organ dysfunction and plasma leakage, while others are uncomplicated. The following were defined as Warning signs- Abdominal pain or tenderness, vomiting, third space fluid accumulation, bleeding manifestation, tiredness, restlessness. Laboratory: Increase in HCT level and low platelet count. Criteria for Severe Dengue includes- Plasma leakage leading to shock (DSS) and/or fluid accumulation with breathing difficulty, bleeding manifestation as evaluated by clinician; Severe organ involvement- Liver: AST or ALT >= 1000, CNS: Altered sensorium, heart and other organs.7

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. Total serum calcium is divided into three forms: 1) Ionized, 50% of the total; 2) Bound to serum albumin, usually 40% of the total; and 3) Complexed with anions such as lactate and phosphate, usually 10% of the total. Ionized calcium (iCa), the active form of calcium is regulated by homeostasis.⁸ 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.⁹

Dengue is fatal unless managed properly. No specific treatment is available and the mainstay of treatment depends on fluid management with careful monitoring of vitals. Evidence for the role of calcium in dengue fever is limited. Low blood calcium levels are found in dengue infection and hypocalcaemia is more evident in severe dengue. The cause for hypocalcaemia is multifactorial. Calcium plays a major role in the immunopathogenesis of dengue; however, the precise mechanism of interactions are not defined properly. Disturbance in the calcium homeostasis is associated with cardiac dysfunction and cardiac arrhythmias seen in dengue as suggested by in vitro studies. Calcium plays a major role in platelet aggregation. Studies regarding supplementation of calcium in dengue have been underpowered and poorly understood to make any firm recommendations. Further studies are needed to confirm the usefulness of maintenance of calcium homeostasis in regulating the cardiac dysfunction, immunopathogenesis and platelet abnormalities related to dengue.10

A study by Uddin et al found that the mean total calcium levels were significantly lower in patients with DHF than in patients with uncomplicated Dengue Fever (DF).¹¹ Constantine et al reported that there is an association between the serum free calcium and the severity of dengue infection.¹² In our present study, the correlation between severity of dengue and the serum free calcium was evaluated.

Objective of the Study

To study the utility of serum free calcium level in predicting the severity of dengue fever.

MATERIALS AND METHODS Source of Data

The study will be done on 100 patients diagnosed to have dengue fever in Bapuji Hospital and Chigateri Government Hospital attached to JJM Medical College will be included in this study.

Method of Collection of Data

100 patients admitted to our hospital serologically confirmed to have dengue infection will be included for the study. Informed written consent will be taken from all the patients. Detailed clinical history will be taken and detailed clinical examination will be done on participating subjects and relevant investigations will be done on the participating subjects.

Sample Size

100

Place of Study

Bapuji Hospital and Chigateri Government Hospital.

Inclusion Criteria

- Patients above the age of 18 years.
- Patients serologically confirmed to have dengue fever by dengue serology.

Exclusion Criteria

Patients with hypertension, diabetes, cardiac and liver diseases and those on anti-hypertensives, anti-arrhythmic drugs, calcium supplements or any other drugs affecting calcium homeostasis were excluded, as these will alter the blood pressure, platelet count and serum calcium level.

Study Design

A cross-sectional study was performed at Jagadguru Jayadeva Murugarajendra Medical College, Davangere, after obtaining ethical clearance from Ethical Committee, JJMMC for a period of 12 months.

Data Analysis

Qualitative data was represented in the form of frequency and percentage. Association between qualitative variables was assessed by Chi-square test.

Quantitative data was represented using Mean and SD. Analysis of quantitative data between three groups was done using ANOVA with Tukey's Post-Hoc multiple comparisons.

To assess the relationship between PLT and Calcium, Pearson's correlation was applied.

P value of < 0.05 was considered as significant.

Statistical analysis was done with IBM SPSS Version 22 for Windows.

RESULTS

The sample size was 100. The majority of the patients were males (n=68, 68%) and females (n=32, 32%) (Figure 1).

Age (In Yrs.)	Frequency	Percent	
20-29	37	37.0	
30-39	23	23.0	
40-49	17	17.0	
50-59	9	9.0	
60-69	9	9.0	
≥ 70	5	5.0	
Total	100	100.0	
Table 1			



Figure 1. Age Distribution of Patients Studied

The average duration of fever was 4.6 days. 35 (35%) patients had mucosal bleeding manifestations (Figure 2).

Bleeding Symptoms	Frequency	Percent	
Haematuria	8	8.0	
Melena	9	9.0	
Oral bleed	11	11.0	
Petechiae	7	7.0	
No	65	65.0	
Total	100	100.0	
Table 2			



Figure 2. Bleeding Manifestations among Patients Studied

26 (26%) patients had history of abdominal pain. 2 (2%) patients had altered sensorium. 4 (4%) patients had restlessness. 7 (7%) unrecordable blood pressure. 67 (67%) patients had NS1 antigen positivity, 18 (18%) patients had IgM positivity and 15 (15%) had both NS1 antigen positivity and IgM positivity. 14 (14%) patients had evidence of pleural effusion. 14 (14%) patients had evidence of ascites.

Average haemoglobin was 12.27 ± 1.21 g/dL and mean haematocrit was 43.08% in our study. Mean serum albumin was 3.78 ± 0.59 mg/dL and 100% had thrombocytopenia.

	HB	PCV	ТС	PLT	Albu	Cal
Mean	12.27	43.08	6186.18	83480.00	3.78	3.92
Std. Deviation	1.21	2.93	2735.30	50175.55	0.59	0.50
Minimum	8.90	38.00	1306.00	9000.00	2.80	2.88
Maximum	14.50	55.00	12400.00	189000.00	5.20	4.61
Table 3						

Based on the new WHO classification, patients were classified into three categories. 37 (37%) patients, 54 (54%) patients and 9 (9%) patients were classified as Dengue fever, Dengue fever with warning signs and Severe dengue (Figure-3). Out of 9 patients with severe dengue, 3 patients succumbed to death. The mortality rate in this study was 3%.

WHO Class	Frequency	Percent	
DF	37	37.0	
DF+WS	54	54.0	
SD	9	9.0	
Total	100	100.0	
Table 4			



Figure 3. WHO Class of Severity of Dengue Fever among Patients Studied

Mean serum calcium in the study was $3.92 \pm 0.50 \text{ mg/dL}$, which is low. Out of 100 patients, 87 (87%) patients had serum free calcium less than 4.5 mg/dL (Figure 4).

Free Calcium	Frequency	Percent	
< 4.5	87	87.0	
≥ 4.5	13	13.0	
Total	100	100.0	
Table 5			



Figure 4. Serum Free Calcium Levels

Free calcium was correlated with WHO class and the severity significantly correlated with the free calcium levels (Figure 5).

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Mean serum free calcium levels in different WHO classes of Dengue fever.

WHO Class	N	Mean Calcium Values	Std. Deviation	ANOVA
DF	37	4.41	0.16	$E_{-114}(2)$
DF+WS	54	3.72	0.34	F=114.02,
SD	9	3.09	0.16	p<0.001
		Table 6		

p value < 0.001**

DF- Dengue Fever, DF + WS- Dengue fever with Warning Signs and SD- Severe Dengue.



Figure 5. Serum Free Calcium in different WHO Class of Dengue Fever

Association was also found between free calcium and platelet count.

	R value	P value		
Pearson Correlation between PLT and Free Calcium	0.961	p<0.000		
Table 7				



DISCUSSION

Ca2+ appears to play a role in the induction of denguespecific 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.¹³

A study conducted in Mexico by Sanchez-Valdez et al on five patients with dengue infection demonstrated that oral

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calcium carbonate and vitamin D3 supplementation significantly increased the number of platelets in patients with dengue infection when compared with a control group.¹⁴ However, randomised controlled trials are presently not there to demonstrate the effectiveness of calcium therapy in the prevention of complications in dengue infection. Oral or IV calcium therapy is not recommended in any guidelines.

Our study has certain limitations. As this study was crosssectional, there was no control group included in the study. Further data on development of complications later on could not be evaluated. Serum calcium samples were not taken on a fixed day after the onset of fever.

CONCLUSION

We conclude that the serum free calcium levels significantly correlated with the severity of DF. The serum free calcium levels were significantly lower in patients with Severe Dengue and Dengue Fever with Warning Signs than in those with Dengue Fever. Free calcium can be used for prognostication of dengue infection severity, but further studies are required to support this.

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REFERENCES

- [1] Rush B. An account of the Bilious remitting Yellow fever, as it appeared in the city of Philadelphia, in the summer and autumn of the year 1793. In: Dobson T, edr. Medical inquiries and observations. Philadelphia: Prichard and Hall 1794: p. 89-100.
- [2] Shukla V, Chandr A. A study of hepatic dysfunction in dengue. J Assoc Physic Ind 2013;61(7):460-1.
- [3] Halstead SB, O'Rourke EJ. Antibody-enhanced dengue virus infection in primate leukocytes. Nature 1977;265(5596):739-41.
- [4] Screaton G, Mongkolsapaya J. T cell responses and dengue haemorrhagic fever. Novartis Found Symp 2006;277:164-71, 171-6, 251-3.
- [5] Green S, Vaughn DW, Kalayanarooj S, et al. Early immune activation in acute dengue illness is related to development of plasma leakage and disease severity. J Infect Dis 1999;179(4):755-62.
- [6] Special Programme for Research and Training in Tropical Diseases, World Health Organization, editors. Dengue: guidelines for diagnosis, treatment, prevention, and control. New edn. Geneva: TDR: World Health Organization, 2009: p. 147.
- [7] Lee LK, Gan VC, Lee VJ, et al. Clinical relevance and discriminatory value of elevated liver aminotransferase levels for dengue severity. PLoS Negl Trop Diseases 2012;6(6):e1676.
- [8] Bushinsky DA, Monk RD. Electrolyte quintet: Calcium. Lancet 1998;352(9124):306-11.
- [9] 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.
- [10] Shivanthan MC, Rajapakse S. Dengue and Calcium. Int J Crit Illn Inj Sci 2014;4(4):314-6.

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- [11] Uddin KN, Musa AKM, Haque WMM, et al. A follow up on biochemical parameters in dengue patients attending BIRDEM hospital. Ibrahim Med Coll J 2008;2(1):25-7.
- [12] Constantine GR, Rajapakse S, Ranasinghe P, et al. Hypocalcemia is associated with disease severity in patients with dengue. J Infect Dev Ctries 2014;8(9):1205-9.
- [13] Chaturvedi P, Saxena V, Dhawan R, et al. Role of calcium in induction of dengue virus-specific helper T cells. Indian J Exp Biol 1995;33(11):809-15.
- [14] Sánchez-Valdéz E, Delgado-Aradillas M, Torres-Martínez JA, et al. Clinical response in patients with dengue fever to oral calcium plus vitamin D administration: study of 5 cases. Proc West Pharmacol Soc 2009;52:14-7.