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ARAŞTIRMA / RESEARCH

Utility of serum free calcium as a predictor of severity in dengue fever

Serum serbest kalsiyumun Dang humması şiddetinin belirleyicisi olarak kullanımı

Jayachandra[1], Kavya S T[1], Sphoorti P Pai[1], Balakrishna A[1]

[1]Bangalore Medical College and Research Institute, Department of Medicine, Bangalore

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Abstract

Purpose: Dengue fever is a worldwide concern due to its considerable mortality and morbidity. The calcium ion plays a critical role in cellular functions and signalling. We evaluated the correlation between severity of dengue and the serum free calcium.

Material and Methods: A cross-sectional study was done in a tertiary care hospital in Bangalore. The 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 145. The mean age was 36.7years and 91(62.7%) were males. 57(39.3%) , 82(56.6%) and 6(4.1%) 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 3.82±0.52 mg/dl. The mean serum calcium(mg/dl) in each class were as follows - Severe Dengue(3.07±0.19) , Dengue Fever with Warning signs(3.70±0.52) and Dengue Fever(4.07±0.40). The presence of hypocalcemia significantly correlated with the severity of dengue.

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, hypocalcemia, serum free calcium.

Öz

Amaç: Dang humması, önemli ölçüde mortalite ve morbiditeye neden olduğundan dünya çapında endişe kaynağıdır. Kalsiyum iyonu hücresel fonksiyonlarda ve sinyalizasyonda kritik bir rol oynamaktadır. Bu çalışmada Dang şiddeti ile serum serbest kalsiyum arasındaki korelasyon değerlendirildi.

Materyal ve Metod: Bangalore'daki , üçüncü basamak sağlık kuruluşunda kesitsel bir çalışma yapıldı. Dang humması hastalarına, NS1 antijen ve/veya IgM antikor testleri kullanılarak teşhis konuldu ve WHO kriterlerine göre sınıflandırıldı. Dang şiddeti ile serum serbest kalsiyum arasındaki ilişki araştırıldı.

Bulgular: Örneklem büyüklüğü 145'dir. Çalışmaya katılan bireylerin yaş ortalaması 36.7 yıl ve 91'i (%62.7) erkektir. Hastalar, Dang humması (DF), Uyarı belirtili Dang humması (DF + WS) ve Şiddetli Dang (SD) olmak üzere sırasıyla 57 (%39.3), 82 (%56.6) ve 6 (%4.1) olarak sınıflandırıldı. Çalışmada serum serbest kalsiyum ortalaması 3.82±0.52 mg / dl idi. Her grubun ortalama serum kalsiyum (mg/dl) değerleri Şiddetli Dang humması için (3.07±0.19), Uyarı işaretli Dang humması için (3.70±0.52) ve Dang humması için (4.07±0.40) şeklindedir. Hipokalsemi varlığı, dang şiddeti ile anlamlı korelasyon göstermiştir.

Tartışma: Çalışma sonucunda serum serbest kalsiyum düzeylerinin dang humması şiddeti ile anlamlı korelasyona sahip olduğu sonucuna vardık. Serum serbest kalsiyum, dang humması şiddetinin bir belirleyicisi olarak kullanılabilir.

Anahtar kelimeler: Dang humması, hipokalsemi, serum serbest kalsiyum.

INTRODUCTION

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 (265-420AD) 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

Yazışma Adresi/Address for Correspondence: Dr. Jayachandra, Bangalore Medical College and Research Institute Department of Medicine, , Bangalore. Email id: sphoortipai@gmail.com
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disease of the devil. The first confirmed case report dates from 1789 and is by Benjamin Rush[1], who coined the term "breakbone fever".

The incidence of dengue has grown dramatically around the world in recent decades. It is estimated that 390 million become infected with dengue per year, of which 96 million manifest clinically[2].It is estimated that 3.9 billion people, in 128 countries, are at risk of infection with dengue viruses[3]. An estimated 500,000 people with severe dengue require hospitalisation each year, a large portion of whom are children. About 2.5% of those affected die. The year 2015 was characterised by large dengue outbreaks worldwide. In 2015, Delhi, India, recorded its worst outbreak since 2006 with over 15000 cases[4]. Dengue virus(DENV) is a single stranded RNA virus of family Flaviviridae, genus flavivirus. There are four serotypes of dengue virus – DENV1, DENV2, DENV3, DENV4. The viral genome is translated to a single chain 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.

Halstead[5] in 1970s proposed the "antibody-dependent immune enhancement theory" for pathogenesis of dengue.Studies have shown 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[6].This skewing of the immune response to the previous dengue serotype is known as "original antigenic sin", which may delay viral control and contribute to a higher peak viraemia and associated severe manifestations[7].This activation of memory CD4+ and CD8+ T cells, sensitized during a previous infection, leads to rapid proliferation and release of proinflammatory cytokines, "cytokine tsunami",particularly TNFα and IFNγ[8]. Insevere dengue the low cytotoxic potential of the T cells fails to obtain early viral control, instead high cytokine-producing cells dominate the response with the excessive pro-inflammatory cytokines causing the tissue damage and plasma leakage.

Dengue can present with a wide spectrum of clinical features, ranging from a simple febrile illness through to severe features of plasma leakage leading to life-threatening shock. Dengue was previously classified into dengue fever and dengue haemorrhagic fever (DHF) of which there were four grades, with DHF III and IV compiling dengue shock syndrome (DSS). In 2009, the WHO[9] reclassified dengue due to difficulty applying the old classification system in clinical situations and increasing reports of severe cases not fitting the criteria for DHF.

The calcium ion plays a critical role in normal cellular function and signalling, regulating diverse physiologic processes such as neuromuscular signalling, cardiac contractility, hormone secretion, and blood coagulation.Total serum calcium exists in three forms: 1)ionized, normally 50% of the total; 2) bound to plasma proteinssuch as albumin, usually 40% of the total; and 3) complexed to anions such as lactate and phosphate, usually 10% of the total. Initial ionized calcium (iCa), the physiologically active form of calcium found in the blood is regulated by homeostasis[10].The total calcium level, therefore, is influenced directly by the serum albumin concentration.Free calcium is a more useful index than total calcium and provides a better indication of calcium status[11].

Hypocalcemia has been commonly found to occur in patients with critical illness, sepsis, trauma.It has been shown to be of prognostic value due to its relation with mortality in critically ill patients[12-16].However, its cause in critically ill patients has not been well defined. Insufficient secretion or inhibited action of parathyroid hormone, decreased vitamin D3 production, and increased calcium deposition, both intra- and extra-cellularly, have all been suggested to be involved in the pathogenesis of hypocalcemia[17]. 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)[18]. Constantine et al. reported that there is an association between the serum free calcium and the severity of dengue infection[19]. In our present study the correlation between severity of dengue and the serum free calcium was evaluated.

MATERIAL AND METHODS

Study population

A cross-sectional study was performed at Bangalore Medical College, Bangalore, Indiaafter obtaining ethical clearance from ethical committee, BMCRI for a period of 24 months. Inpatients with

confirmed dengue infection were recruited for the study, after written consent was obtained. Patients with hypertension, diabetes, cardiac and liver 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, platelet count, liver enzymes and serum calcium levels.

Definitions

A probable case of dengue was diagnosed according to the World Health Organization (WHO) criteria[9]. Confirmation of diagnosis was done with one of the following laboratory tests: Serum dengue NS1 (non-structural protein 1) antigen or IgM antibody. Dengue infection was diagnosed and classified in to three stages according to the WHO criteria as follows: Dengue without warning signs, Dengue with warning signs, Severe dengue.The following were defined as Warning signs - Abdominal pain or tenderness, Persistent vomiting, Clinical fluid accumulation, Mucosal bleed, Lethargy, restlessness, Liver enlargement>2 cm, Laboratory: increase in HCT concurrent with rapid decrease in platelet count. Criteria for Severe Dengue were as follows - Severe plasma leakage leading to shock (DSS) and/or fluid accumulation with respiratory distress; Severe bleeding as evaluated by clinician; Severe organ involvement - Liver: AST or ALT >=1000, CNS: Impaired consciousness, Heart and other organs.

Data collection

Details of the patient and presenting complaints like fever duration, presence of giddiness, abdominal pain, bleeding manifestations, altered sensorium, restlessness etc. were collected interviewer administered questionnaire. The clinical parameters recorded were pertaining to evidence of hemodynamic instability(pulse volume, blood pressure, pulse pressure, presence of cold extremities) evidence of fluid leakage (pleural effusion and ascitis).

In addition, the following investigations were performed in first 24 hours of admission: Haemoglobin, Total leucocyte count, platelet count, packed cell volume, Renal function tests - urea, creatinine, liver function tests - S.Bilirubin, S.Albumin, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), total calcium and serum free calcium levels.

Hypocalcemia was defined as serum free calcium level of <4.5mg/dl. Blood was collected in an EDTA tube for haemoglobin, total leucocyte count, platelet count and packed cell volume and analysed by automated analyser. For other investigations blood was collected in plain tubes. Blood for serum ionised calcium was collected without applying tourniquet. Roche 9180 Electrolyte Analyser for serum free calcium measurement.

Statistical analysis

Data were analysed using SPSS version 22 statistical software package. The significance of the differences between proportions and means were performed using univariate correlation analysis for continuous variables and student's t-test or anova for categorical data columns. In addition, Bonferroni's test was used to conduct post hoc analysis for the categorical columns. Charts and tables were generated with the help of Microsoft Excel.

RESULTS

The sample size was 145.The majority of the patients were males (n =91, 62.7%), and mean age was 36.7±12.6 years(Figure 1). The average duration of fever was 4.1days. 46 (31.7%) patients had mucosal bleeding manifestations (Figure 2). 51 (35.1%) patients had history of abdominal pain 1patient had altered sensorium.

Four (2.7%) patients had restlessness. 6(4.1%) patients had cold extremities with unrecordable blood pressure. 131 (90.3%) patients had NS1 antigen positivity and 24 (16.6%) patients had IgM positivity. 43 (29.6%) patients had evidence of pleural effusion.37 (25.5%) patients had evidence of ascites. Average haemoglobin was 13.6±1.8g/dl and mean haematocrit was 41.4% in our study. 60(41.4%) patients had leucopenia and all the patients(100%) had thrombocytopenia.

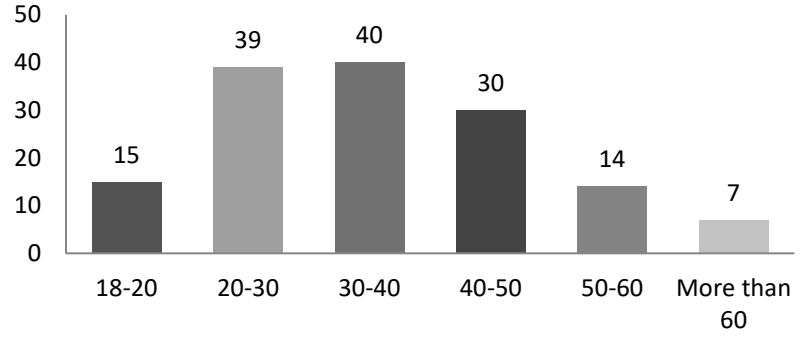

Figure 1. Age distribution of patients

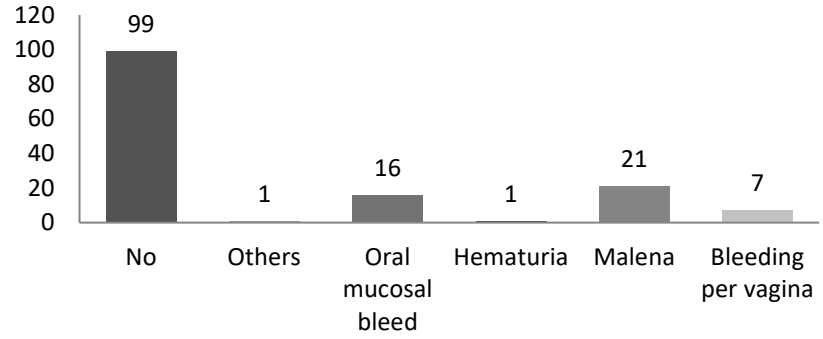

Figure 2. Bleeding manifestations among patients

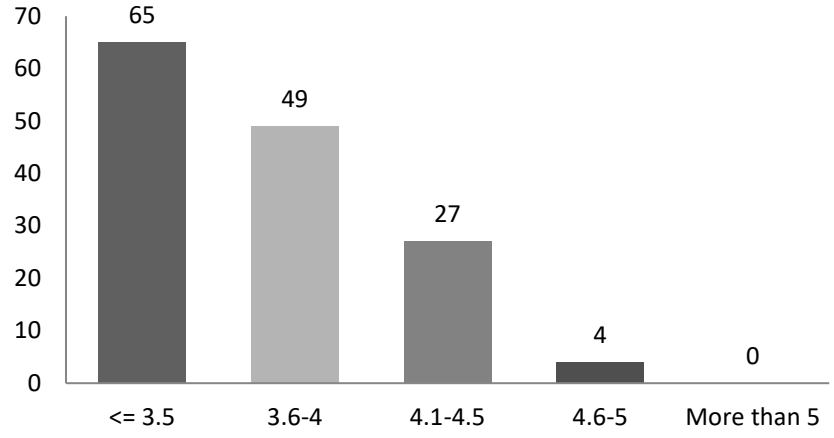

Figure 3. Serum Albumin levels in patients

The mean creatinine in males was 0.73 mg/dl and in females was 0.86 mg/dl. The average total bilirubin was 0.77±0.59 mg/dl and mean serum albumin was 3.63±0.50 mg/dl. 65 (44.8%) of patients had hypoalbuminemia(Figure 3). Ninety nine (68.2%) patients had raised liver enzymes Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) out of which 3 patients had AST and ALT >1000 U/L. Based on the new WHO classification patients were classified into three categories. 57 (39.3%) patients, 82 (56.6%) patients and 6 (4.1%) patients were classified as Dengue

fever, Dengue fever with warning signs and Severe dengue (Figure 4).

Out of the 6 patients with severe dengue 5 patients succumbed to death. The mortality rate in this study was 3.4%.Mean serum calcium in the study was 3.82±0.52 mg/dl which is low. Out of 145 patients 130 (89.6%) patients had serum free calcium less than 4.5 mg/dl (Figure 5). Free calcium was correlated with WHO class and the severity significantly correlated with the free calcium levels (Table 1, Figure 6). Association was also found between free calcium and individual parameters like AST, ALT, creatinine, haematocrit, platelet count and blood pressure (Table 2).

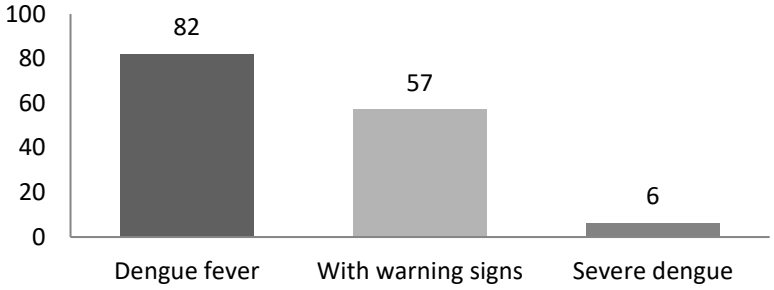

Figure 4. WHO class of severity of Dengue fever among patients

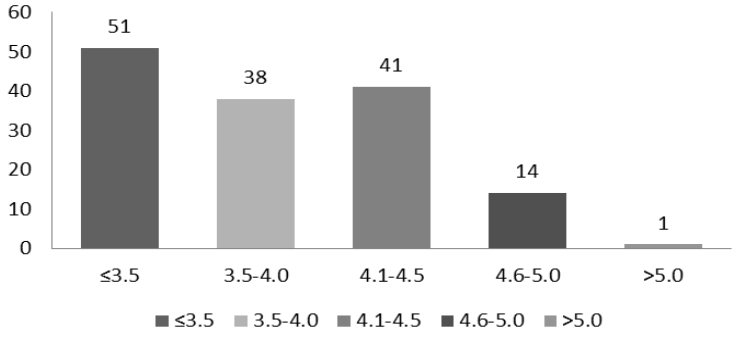

Figure 5. Serum free calcium levels in patients

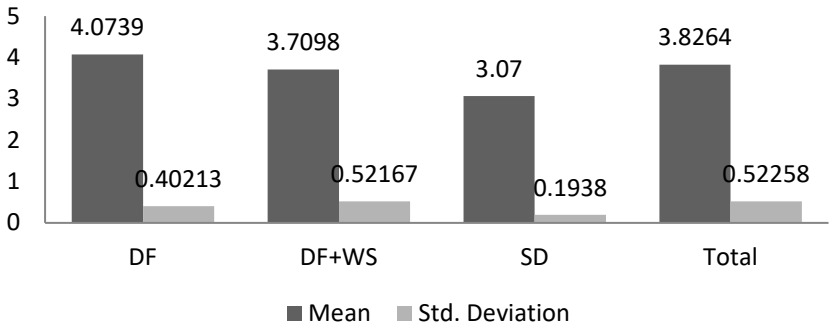

Figure 6. Serum free calcium in different WHO class of dengue fever

Table 1.Mean Serum free calcium levels in different WHO classes of Dengue fever

WHO class	Mean Serum free Calcium	Std. Deviation	N
DF	4.0739	0.40213	57
DF+WS	3.7098	0.52167	82
SD	3.0700	0.19380	6
Total	3.8264	0.52258	145

p value <0.001** DF-Dengue Fever, DF+WS-Dengue fever with Warning Signs and SD-Severe dengue

Table 2. Correlation of other parameters with serum free calcium

	Mean	Std. Deviation	correlation	P value
Free Calcium	3.8264	.52258	1	
AST	126.35	372.099	-0.201*	0.016
ALT	98.88	364.924	-0.192*	0.021
Creatinine	.783	.4367	-0.295**	0.000
HCT	41.350	6.2253	-0.097	0.245
PLT	47903.45	28356.932	0.197	0.017
BP	115.860	16.8316	0.580	0.492

DISCUSSION

It is now evident that dengue is a worldwide concern. However, almost 75% of the global population exposed to dengue live in Asia-Pacific. 1.3 billion of these at-risk individuals live in ten dengue endemic countries in South East Asia[20]. Dengue is a rapidly emerging disease in India and has been prevalent for almost 230 years. India faced a major outbreak in 2015 and recorded 99913 cases and 220 deaths according to the National Vector Borne Disease Control Programme[21]. Rapid triage and effective therapy is very important in management of dengue. There is a need for newer modalities of treatment as the atypical manifestations with severe multi-organ involvement are increasingly being reported. Assessment of prognosis is also very important.

In this study it was noted that free serum calcium levels significantly correlated with the severity of dengue. Mean free calcium was found to be lower with increasing severity. Several causes for low blood calcium levels have been suggested, including reduced Na+-K adenosine triphosphatase (ATPase) activity, reduced Ca2+-ATPase activity, acquired parathyroid hormone deficiency, renal one-alpha hydroxylase insufficiency, reduced dietary vitamin D intake, and reduced dietary calcium intake[22]. 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[23]. Though there have been many studies on low serum calcium levels in sepsis, the role of calcium in dengue has not been elucidated completely.The exact mechanisms for development of hypocalcemia in severe dengue infections also requires further study.

Further studies are required to determine whether the presence of hypocalcemia can be used as a prognostic indicator to predict disease severity. The study by Adikari et al.[24] concluded that serum ionized calcium level was significantly reduced in majority of patients with severe dengue infecton within first 24 hours of onset of severe dengue clinical criteria. This was similar to the results of our study.

There have not been many studies to assess the effects of calcium supplementation for dengue patients. A study conducted in Mexico by Sanchez-Valdez et al.[25] on five patients with dengue infection demonstrated that oral calcium carbonate and vitamin D3 supplementation significantly increased the number of platelets in patients with dengue infection when compared with a control group.A study done in Pune compared the levels of Vitamin D in patients having dengue infection with healthy individuals and found that it was significantly higher in patients with dengue[26]. However, randomized control 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. Other biomarkers for severe dengue like Interleukins have been studied. Malavige et al[27]. studied serum Interleukin-10 (IL-10) as a marker of

SDI but concluded as unsuitable to be used as a robust biomarker because of its poor discriminatory value between severe dengue and non-severe dengue patients.

Our study has certain limitations. As this study was cross-sectional, 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. There was no single method for diagnosis of dengue and different tests like NS1 or IgM was considered, hence having different sensitivity and specificity. PCR could not be done due to financial constraints.

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 prognostification of dengue infection severity but further studies are required to support this. In addition, studies are needed to analyse the effect of supplementation of calcium in dengue fever for reduction of severity.

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