



Serum electrolytes and their prognostic value in Dengue fever: A study from South India

Dr Utkarsh, Dr M Mahesh, Madhumitha M, Dr Akshay P Rav

Junior resident dept of medicine Jss medical college JSS AHER Mysuru

Professor of medicine JSS medical college JSSAHER Mysuru

Junior resident MS R medical college Bengaluru

Junior resident JSS medical college JSSAHER Mysuru

corresponding author:Dr M Mahesh

doctmahesh@mail.com

ABSTRACT

Background: In Dengue infection plasma leakage, shock and bleeding may result from derangement of coagulation system and activation of endothelial cells due to various inflammatory mediators such as cytokines and chemokines. Hence this study was conducted.

Methods: In the present study, 95 confirmed dengue cases aged above 16 years were included, for a period of 18 months in JSS Hospital and Medical College, Mysore.

Results: Mean age was 36.17 ± 15.842 years. 62.1% were males and 37.9% were females. Mean sodium was 135.4 ± 5.5 mEq/L, mean potassium was 4.23 ± 0.57 mEq/L and mean calcium was 9.01 ± 0.41 mg/dl. 36.8% had Hyponatremia, 7.4% had Hypokalemia, 3.2% had Hyperkalemia and 3.2% had Hypocalcaemia. 60% had Dengue Fever, 17.9% had DHF I, 8.4% had DHF II and DHF III respectively and 5.3% had DHF IV. Mean Sodium levels among subjects with DHF III was 129.0 ± 9.8 mEq/L and among subjects with DHF IV was 131.2 ± 9.1 mEq/L. There was significant difference in mean sodium levels compared to severity of dengue illness. Among subjects with DF, 1.8% had Hypocalcaemia and among subjects with DHF II, 25% had Hypocalcaemia. There was significant association between diagnosis and calcium levels.

Conclusions: From the study it can be concluded that with increase in Severity of Dengue fever, there was decrease in Serum sodium and Serum Calcium levels. However, potassium levels were not affected with the severity of Dengue fever. This suggests that apart from haematological derangement, there is serum electrolyte imbalance with the severity of Dengue fever.

Keywords: dengue, electrolyte, hyponatremia, hypocalcemia

INTRODUCTION:

Dengue is a mosquito-borne viral disease that has rapidly spread in all regions of WHO in recent years. Dengue virus is transmitted by female mosquitoes mainly of the species *Aedes aegypti* and, to a lesser extent, *Aedes albopictus*. Dengue is widespread throughout the tropics, with local variations in risk influenced by rainfall, temperature and unplanned rapid urbanization¹.

Dengue fever is caused by infection with one of the four serotypes of dengue virus (DENV) which is an arthropod borne single stranded RNA virus of genus *Flavivirus*². It is comprised of four closely related but antigenically distinct serotypes, DENV1, DENV2, DENV3 and DENV4. Infection with one dengue serotype confers lifelong homotypic immunity to that serotype and a very brief period of partial heterotypic immunity to all other serotypes, but a person can eventually be infected by all four serotypes³. Subsequent infections by other serotypes increase the risk of developing severe dengue¹.

Severe dengue affects most Asian and Latin American countries and has become a leading cause of hospitalization and death among children and adults in these regions¹. The incidence of dengue has grown dramatically around the world in recent decades^{4,5}. All four serotypes have been isolated in India, DENV1 and DENV2 serotypes are wide spread⁶. Dengue is endemic in 31 states / UTs. During 2013 about 74168 cases were reported with 168

deaths, the highest number of cases were reported from Punjab followed by Tamil Nadu, Gujarat, Kerala and Andhra Pradesh ⁷.

Dengue fever is a severe, flu-like illness that affects infants, young children and adults, but seldom causes death. Dengue should be suspected when a high fever (40°C/104°F) is accompanied by 2 of the following symptoms: severe headache, pain behind the eyes, muscle and joint pains, nausea, vomiting, swollen glands or rash. Symptoms usually last for 2–7 days, after an incubation period of 4–10 days after the bite from an infected mosquito¹.

Severe dengue (Dengue Hemorrhagic Fever and Dengue Shock Syndrome) is a potentially deadly complication due to plasma leaking, fluid accumulation, respiratory distress, severe bleeding, or organ impairment. Warning signs occur 3–7 days after the first symptoms in conjunction with a decrease in temperature (below 38°C/100°F) and include: severe abdominal pain, persistent vomiting, rapid breathing, bleeding gums, fatigue, restlessness and blood in vomit. The next 24–48 hours of the critical stage can be lethal; proper medical care is needed to avoid complications and risk of death. Currently the treatment of Dengue infection depends upon the clinical presentation and associated complications such as plasma leakage and electrolyte abnormalities¹.

In Dengue infection plasma leakage, shock and bleeding may result from derangement of coagulation system and activation of endothelial cells due to various inflammatory mediators such as cytokines and chemokines. Hence this study was conducted in the present institute to determine the electrolyte abnormalities among dengue patients.

OBJECTIVE OF THE STUDY:

- To determine the prevalence of electrolyte abnormalities among Dengue Infection.
- To determine association between serum electrolyte abnormalities with severity of illness.

METHODOLOGY

- **STUDY DESIGN:** Cross-sectional study
- **STUDY DURATION:** 18 months (November 2019 to April 2021)
- **STUDY AREA:** Kempegowda Institute of Medical Science, Bangalore.
- **STUDY PARTICIPANTS:** Patients with confirmed Dengue Infection (by Dengue IGM Antibody by ELISA) admitted to JSS Hospital, Mysuru, General Medicine OPD/IPD.
- **INCLUSION CRITERIA**
 1. Adult patients (greater than 16 years of age) with confirmed dengue infection (by Dengue IGM antibody by ELISA) admitted to JSS Hospital Mysuru
- **EXCLUSION CRITERIA**
 1. Patients with Diabetes Ketoacidosis
 2. Chronic Kidney Disease.
 3. Pregnancy.
 4. Patients with Addison's disease
 5. Patients with Conn's Disease
 6. Patients on diuretic therapy
 7. Coronary Artery Disease
 8. Chronic Obstructive Pulmonary Disease
 9. Malignancies

ESTIMATION OF SAMPLE SIZE:

The sample size for this study was calculated at 5% level of significance, 25% relative precision and 40% prevalence based upon a previously done study⁸. The sample size from above calculation comes to be 95.

METHOD OF DATA COLLECTION:

Data was collected by using a structured questionnaire. It consisted of General profile, clinical profile and laboratory profile of Dengue Patients. All patients underwent brief clinical examination which includes measurement of temperature, pulse, blood pressure. Hydration status was assessed clinically by examination of the tongue for moistness, skin turgor and urine output and Skin Examination to look for any bleeding manifestations. Clinical evidence of any plasma leakage will also be looked for (ascites, pleural effusion). Based upon above examination and thrombocytopenia patients were classified into either uncomplicated dengue fever or dengue hemorrhagic fever. Other laboratory parameters such as platelets, haematocrit and serum electrolytes such as Serum Sodium, Potassium and Calcium levels were measured. Blood samples were collected into an EDTA-coated Vacutainer tube for haematological investigations and serological tests and plain vacutainer tube for biochemical tests.

METHOD OF DETERMINATION OF CALCIUM

Calcium Estimation was based on Colorimetric method. The specific binding of Arsenazo III and calcium at acid pH with the resulting shift in the absorption wavelength of the complex. The intensity of the chromophore formed is proportional to the concentration of total calcium in the sample.

REFERENCE VALUES: Adults (12-60 years) - 8.4 - 10.2 mg/dL (2.1 - 2.5 mmol/L)

METHOD OF DETERMINATION OF SODIUM AND POTASSIUM WAS DONE BY ATOMIC ABSORPTION SPECTROSCOPY.

ELISA – Dengue NS-1 antigen:

The blood samples obtained were tested for dengue NS-1 antigen using ‘Panbio diagnostics’ manufactured by J.Mitra and co. In this test, dengue anti NS1 antibody is quoted to the wells and if the patient’s serum contains dengue NS1 antigen it binds to anti NS1

antibody and produces a colour change. Cut off optical density (OD) was 0.418, the ratio of OD of the test sample with cut-off OD was taken and a ratio of more than 1 was taken as positive.

ELISA – Dengue IgM

Anti-dengue IgM antibodies were tested by MAC ELISA. The wells are coated with anti-human IgM which capture IgM antibodies in the patient's serum. DENV antigen was then added and the wells were washed to remove unbound antigen. Biotinylated flavivirus cross reactive antibody was then added along with chromogen and colour change was observed. The cut off OD considered was 0.441. Then the ratio of test sample OD and cut off OD was taken and values more than 1.5 was taken as positive.

Dengue Haemorrhagic Fever was Graded:

Grade I: Fever and nonspecific constitutional symptoms, with a positive tourniquet test being the only hemorrhagic manifestation

Grade II: Grade I along with spontaneous bleeding

Grade III: Circulatory failure manifested as rapid/ weak pulse with cold skin and restlessness and hypotension

Grade IV: Profound shock with nondetectable pulse or blood pressure Grade III and grade IV were classified as Dengue Shock Syndrome

STATISTICAL ANALYSIS:

Data was entered into Microsoft excel data sheet and was analyzed using SPSS 23 version software. Categorical data was represented in the form of Frequencies and proportions. Chi-square test was used as test of significance for qualitative data. Continuous data was represented as mean and standard deviation. ANOVA (Analysis of Variance) was

the test of significance to identify the mean difference between more than two groups for quantitative data. P value of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

RESULTS:

Mean age was 36.17 ± 15.842 years. Majority of subjects were in the age group 21 to 30 years (33.7%). In the study 62.1% were males and 37.9% were females. In the study most common symptom on presentation was Myalgia (57.9%), followed by Arthralgia (54.7%) and other symptoms as shown in above table. 30.5% were febrile, 30.5% had tachycardia, 10.5% had hypotension, 34.7% had ascites, 20% had pleural effusion, 32.6% had Positive Tourniquet Test and 12.6% had skin Turgor.

Mean platelet count was 0.7 ± 0.8 lakhs and mean Hematocrit was 43.4 ± 6.7 . Mean sodium was 135.4 ± 5.5 mEq/L, mean potassium was 4.23 ± 0.57 mEq/L and mean calcium was 9.01 ± 0.41 mg/dl. 60% had Dengue Fever, 17.9% had DHF I, 8.4% had DHF II and DHF III respectively and 5.3% had DHF IV.

In the study 36.8% had Hyponatremia, 7.4% had Hypokalaemia, 3.2% had Hyperkalaemia and 3.2% had Hypocalcaemia. In the study incidence of Hyponatremia was highest in subjects with DHF III and DHF IV (62.5% and 60% respectively). There was significant association between severity of dengue and sodium levels. In the study highest incidence of Hypokalemia was seen in subjects with DHF IV (40%) and highest incidence of Hyperkalemia was seen in subjects with DHF I. However, there was no significant association between diagnosis and potassium levels. .8% had Hypocalcaemia and among subjects with DHF II, 25% had Hypocalcaemia. There was significant association between diagnosis and calcium levels.

Mean Sodium levels among subjects with DHF III was 129.0 ± 9.8 mEq/L and among subjects with DHF IV was 131.2 ± 9.1 mEq/L. Other diagnosis of dengue showed normal levels of sodium. There was significant difference in mean sodium levels compared to severity of dengue illness. there was no significant difference in mean Potassium levels with respect to severity of dengue illness. there was significant difference in mean Calcium levels with respect to severity of dengue illness. Mean Calcium decreased with increase in severity of disease.

DISCUSSION:

The mean age of the study population was 36.17 ± 15.842 years. Majority of subjects were in the age group 21 to 30 years (33.7%). This is comparable with the studies done by Nandini et al⁹, Singh R et al¹⁰, Kauser MM et al¹¹, Ashwini Kumar et al¹², Oza JR et al¹³, Mohd. Yaseen et al¹⁴, Padyana M et al¹⁵ implying that dengue is affecting the middle age and working population.

It was observed that Dengue was common among Males compared to Females in almost all the studies⁹⁻¹⁴ and similar observation was made in the present study. However, in the study by Padyana M et al¹⁵, slight female predominance was observed (51%).

In the study most common symptom on presentation was Myalgia (57.9%), followed by Arthralgia (54.7%) and other symptoms as shown in above table. In the study by Agrawal VK¹⁶, 100% of them had fever, 33.5% had Arthralgia, 88% had myalgia, 38% had headache and 30.8% had hemorrhagic manifestations such as rashes and Malena. Similarly, in the studies by Malavige et al¹⁷ and NP Singh et al¹⁰⁸, varied presentations of dengue were observed. Hence Clinical presentation depends on severity and timing of admission.

In the study 60% had Dengue Fever, 17.9% had DHF I, 8.4% had DHF II and DHF III respectively and 5.3% had DHF IV. In the study by MR Rajalekshmy et al¹⁹, 54.73% had DF, 1.05% had DHF I, 43.16% had DHF II, 1.05% had DHF III and 0% had DHF IV.

Thrombocytopenia has always been one of the criteria used by WHO guidelines as a potential indicator of clinical severity. In the most recent 2009 WHO guidelines, the definitions generally describe a rapid decline in platelet count or a platelet count less than 150,000 per microliter of blood. The mechanisms involved in thrombocytopenia and bleeding during DENV infection are not fully understood. Several hypotheses have been suggested to elucidate the mechanism involved. In this context, DENV could directly or indirectly affect bone marrow progenitor cells by inhibiting their function to reduce the proliferative capacity of hematopoietic cells. The mean platelet count was 0.7 ± 0.8 lakhs /cumm in the present study and there was decrease in platelet count with respect to severity of Dengue. Shekar EC et al²⁰ and Chuansumrit A et al²¹ observed significant difference in Mean platelet with respect to Severity of Dengue, which was similar to the present study.

The increase in Hematocrit in dengue is due to hemoconcentration attributed to plasma leakage induced by cytokine-mediated increase in vascular permeability and damage to vascular endothelium. Cytokines are produced by DENV infected Monocytes, B lymphocytes, and mast cells. Endothelial cell dysfunction by virus also leads to increased capillary permeability. This phase of plasma leakage is the critical phase, the onset of which (marked by circulatory and perfusion changes leading to shock) can be predicted with the rise of Hematocrit 10–15% above the baseline value. This is considered a significant predictor of severe disease. The mean Hematocrit was 37.82% (range 21.4-53.5%). Comparison of Hematocrit between DF, DHF1, DHF2 and DSS did not reveal any statistically significant P value. ($P = 0.101$). Our findings were similar to studies by Shekar EC et al²⁰ and Chuansumrit A et al²¹ with respect to Hematocrit. Higher Hematocrit values were observed in the study by Srividya et al. This was because of the older classification used in the study as Dengue without warning signs, Dengue with warning signs and severe dengue.

Mean sodium was 135.4 ± 5.5 mEq/L, mean potassium was 4.23 ± 0.57 mEq/L and mean calcium was 9.01 ± 0.41 mg/dl. In the present study significant difference was observed in mean Sodium and Calcium with respect to Type of Dengue. Mean sodium and Calcium were reduced with increase in Severity of Dengue. However, there was no significant difference in mean Potassium with respect to Severity of Dengue. In the study by MR Rajalekshmy et al¹⁹, mean serum sodium level was found to be 135.92 ± 5.53 mEq/l and mean serum potassium was 3.84 ± 0.55 mEq/l in the study subjects.

In a study done by Prakash Ram Relwani et al²², mean serum sodium level was found to be 136.01 ± 5.10 mEq/l and mean serum potassium was 3.91 ± 0.48 mEq/l. Hyponatremia (45.33%) was more commonly observed than hypernatremia (3.33%). Hypokalaemia (10.60%) was more commonly observed than hyperkalaemia (3.33%). MR Rajalekshmy et al¹⁹ almost two-third of the patients (64 %) had normal serum sodium level (≥ 135 mEq/l). 26 out of 95 patients (28 %) had mild hyponatraemia (130 - 134 mEq/l), 6 patients (~6%) had moderate hyponatraemia (120 - 129 mEq/l) and 2 patients (~ 2%) had severe hyponatraemia (< 120 mEq/l).

In our study, 63 patients (66 %) had normal serum potassium level (> 3.5 mEq/l). 28 patients (30%) had mild hypokalaemia (3 - 3.5 mEq/l) and 4 patients (~4%) had moderate hypokalaemia (2.5 - 2.9 mEq/l). No patient had severe hypokalaemia (< 2.5 mEq/l). A study done in a paediatric population by Lumpaopong A et al²³ in Thailand showed the prevalence of hyponatraemia to be 61% in DF and 72% in DHF. The prevalence of hypokalaemia was only 14% in DF and 17% in DHF. Hyponatraemia was attributed to increased antidiuretic hormone levels, whereas hypokalaemia was due to increased renal excretion of potassium due to activation of renin-angiotensin aldosterone mechanism secondary to volume depletion. A similar study by Joshi R et al⁸ done in a paediatric population with dengue fever in a tertiary care centre in Mumbai found the prevalence of hyponatraemia to be 40.3%.

A study done by Rose JC et al²⁴ showed the prevalence of hyponatraemia to be 58%, but hypokalaemia was not found among the study patients. A study done by Reddy AA et al.,²⁵ found that, out of 99 subjects, 63 showed hyponatraemia. Among these, 33 patients showed mild hyponatraemia, 12 had moderate and 18 had severe hyponatraemia, and 2 patients developed complications.

Constantine GR et al²⁶ in their study found that there is significant correlation between dengue severity and Serum Ca²⁺ levels. Madura Adikari et al²⁷ found that 52(85%) showed hypocalcaemia during the first 24 hours of onset of severe dengue infection. Mean ionized calcium level of the population was 0.96 mmol/L and the range being 0.53-1.48 mmol/L. There was major reduction in serum calcium level within the first 24 hours of the onset of severe dengue clinical criteria.

CONCLUSION:

From the study it can be concluded that with increase in Severity of Dengue fever there was decrease in Serum sodium and Serum Calcium levels. However, potassium levels were not affected with the severity of Dengue fever. This suggests that apart from haematological derangement there is serum electrolyte imbalance with the severity of Dengue fever.

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Table 1: PATIENT CHARACTERISTICS

PATIENT CHARACTERISTICS		FREQUENCY	PERCENT
Age	18 to 20 years	14	14.7%
	21 to 30 years	32	33.7%
	31 to 40 years	21	22.1%

	41 to 50 years	9	9.5%
	51 to 60 years	11	11.6%
	>60 years	8	8.4%
Gender	Male	59	62.1%
	Female	36	37.9%
Symptoms	Fever	29	30.5%
	Arthralgia	52	54.7%
	Myalgia	55	57.9%
	Headache	39	41.1%
	Retro orbital pain	26	27.4%
	Hemorrhagic manifestations	17	17.9%
Urine output	Adequate	79	83.2%
	Decreased	16	16.8%
Tongue	Dry	47	49.5%
	Moist	48	50.5%
Signs	Febrile	29	30.5%
	Tachycardia	29	30.5%
	Hypotension	10	10.5%
	Ascites	33	34.7%
	Pleural effusion	19	20.0%
	Positive tourniquet test	31	32.6%
	Skin turgor (poor)	12	12.6%
Elisa NS1	Negative	42	44.2%
	Positive	53	55.8%
Elisa IgM	Positive	95	100.0%
Diagnosis	DF	57	60.0%
	DHF I	17	17.9%
	DHF II	8	8.4%

	DHF III	8	8.4%
	DHF IV	5	5.3%

Table 2: Laboratory parameters

Laboratory parameters		Mean	S. D
CBC profile	Platelet	0.7	0.8
	Hematocrit	43.4	6.7
Serum electrolytes	Sodium	135.4	5.5
	Potassium	4.23	0.57
	Calcium	9.01	0.41

Table 3: Abnormalities in serum electrolytes

Serum electrolytes		FREQUENCY	PERCENT
Sodium	<136 (Hyponatremia)	35	36.8%
	136 to 145 (Normal)	60	63.2%
Potassium	Hypokalaemia (<3.5)	7	7.4%
	Normal (3.5 to 5.1)	85	89.5%
	Hyperkalaemia (>5.1)	3	3.2%
Calcium	Hypocalcaemia (<8.4)	3	3.2%
	Normal (8.4 to 10.2)	92	96.8%

Table 4: Association of Serum electrolytes with the final diagnosis

Serum electrolytes	Diagnosis	Mean	SD	P value
	DF	136.0	4.3	0.001*
	DHF I	137.5	3.6	

Sodium	DHF II	135.9	3.6	
	DHF III	129.0	9.8	
	DHF IV	131.2	9.1	
Potassium	DF	4.25	0.60	0.908
	DHF I	4.22	0.44	
	DHF II	4.23	0.62	
	DHF III	4.30	0.35	
	DHF IV	4.00	0.88	
Calcium	DF	9.019	0.371	0.019
	DHF I	9.206	0.423	
	DHF II	8.737	0.495	
	DHF III	8.913	0.405	
	DHF IV	8.680	0.044	