

A Study On Correlation Of Serum Calcium With Severity Of Dengue Fever Admitted At Tertiary Care Hospital

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INTRODUCTION

MATERIALS AND METHODS:

Study design: A case series study

Study period: 1.5 years

Place of study: The study is planned to be conducted at the department of General medicine, SNMC and HSK HOSPITAL BAGALKOT.

This study was conducted among 140 patients who are admitted with fever in SNMC Medical College, Bagalkot, in department of General Medicine. Study was done for 1.5 to 2 years

INCLUSION CRITERIA:

Cases diagnosed according to WHO criteria.

Patients with,

1. Dengue NS1 antigen positive

2. IgM ELISA

Patients above 18 yrs of age

EXCLUSION CRITERIA:

Patients with;

- Hypertension
- Diabetes and cardiac disease
- Malaria
- Calcium supplements
- Those on antihypertensives/anti arrhythmic agents
- Any drugs affecting calcium homeostasis
- Those who doesn't give consent.
- Malabsorption syndrome
- Renal dysfunction

Present study will be carried out in the department of General medicine, S NIJALINGAPPA MEDICAL COLLEGE AND HANAGAL SHRI KUMERASHWARA HOSPITAL, NAVANAGAR, BAGALKOT.

After obtaining approval and clearance from the institutional ethical committee, the patient fulfilling the inclusion criteria will be enrolled for the study after obtaining informed consent. total of 140 patient who diagnosed as Dengue Fever shall be taken as cases. venous blood samples (into 2ml of EDTA tube and another 2ml in plain tube) for required blood investigation and other required investigation is done.

Data collection:

1. Informed consent will be taken for participation in the study.

2. A questionnaire will be used to collect data including symptoms¹⁵(fever, headache, retro-orbital pain, arthralgia, myalgia, rash, and bleeding manifestations), evidence of fluid leakage(pleural effusion and

ascites), pulse rate, and systolic and diastolic blood pressure.

Sample: blood samples for the estimation of serum calcium were drawn on the day of admission.

Confirmatory tests:

NS1 antigen detection

IgM ELISA

All probable cases of dengue were diagnosed according to World Health Organization (WHO) criteria.⁴

Confirmation of diagnosis was done with one of the following laboratory tests: IgM antibody, NS1 Ag positive.

CRITERIA FOR DENGUE WITHOUT WARNING SIGNS:

Living in/travel to dengue endemic area. Fever and two of the following criteria: Nausea, vomiting, rash, aches, and pains, leucopenia, any warning sign, laboratory confirmed dengue (when warning signs are absent)

CRITERIA FOR DENGUE WITH WARNING SIGNS:

Warning signs: Abdominal pain or tenderness, Persistent vomiting, clinical fluid accumulation, mucosal bleed, lethargy; restlessness, liver enlargement >2cm, Laboratory: Increase in haematocrit concurrent with rapid decrease in platelet count.

CRITERIA FOR SEVERE DENGUE:

1. Severe plasma leakage leading to: Shock (DSS), Fluid accumulation with respiratory distress
2. Severe bleeding as evaluated by clinician
3. Severe organ involvement: Liver: AST or ALT ≥ 1000 ; CNS: Impaired Consciousness; Heart; and other organs

The following blood investigations will be performed on the day of admission; haemoglobin (Hb); total leukocyte count (TLC); platelet count; packed cell volume (PCV); liver function tests and serum calcium levels.

Other investigations such as chest X-ray (CXR), ultrasonography (USG), and echocardiography (ECHO) will be done based on clinical presentation as and when required. These investigations will be done in case any complication was suspected. Pleural effusion will be diagnosed by chest radiography, and abdominal sonography will be done to rule out ascites and to note the gall bladder wall thickness. Myocarditis will be diagnosed by two dimensional ECHO.

Blood will be collected in EDTA tube for Hb, TLC, platelet count, and PCV will be analyzed by an automated analyser. For other investigations, blood will be collected in plain tubes.

Other than routine investigations will be paid to avoid burden on patient.

Expected outcome: Early estimation of serum calcium in dengue fever will help in analyzing the severity of dengue infection, approximate duration of hospital stay.

Investigations: required investigation done on the patient are

Hb g/dl:

TC cells/mm³:

Platelets lakhs/mm³:

RBCs million/mm³:

ESR mm/hr:

PCV %:

Glucose (RBS) mg/dl:

Urea mg/dl:

Creatinine mg/dl:

Na⁺ mEq/l:

K⁺ mEq/l:

Ca²⁺ mEq/l:

Urine routine & Microscopy:

LFT:

TB :

DB :

IB :

TP :

SGOT:

SGPT:

DENGUE SEROLOGY: NS1 Ag :

IgM ANTIBODY:

MP antigen :

ULTRASONOGRAM OF ABDOMEN: (if necessary)

ECG:

X RAY CHEST: (if necessary)

ECHO CARDIOGRAM: (if necessary)

ESTIMATION OF CALCIUM:

Method: Arsenazo

Mode: Endpoint

Principle:

Arsenazo combines with calcium ions at PH 6.5 to form a coloured chromophore. The color produced is directly proportional to the concentration of calcium in the sample which is measured at 650nm.

Reagent composition

- Calcium standard: 10mg/dl
- Arsenazo III
- Buffer

Procedure

The assay was performed after calibration.

10µl of serum was mixed with 1ml of reagent, mixed and incubated for 10 minutes at room temperature and absorbance of standard and sample read against reagent blank at 650nm.

Calculation

Serum Calcium (mg/dL) = $\frac{(\text{Abs.T}) - (\text{Abs.B})}{(\text{Abs.S}) - (\text{Abs.B})} \times 10$

Reference Range

Serum calcium 8.5-10.5mg/dl

Linearity

The kit is linear up to 16mg/dl

DIAGNOSIS OF DENGUE:

1. IgM-capture MAC-ELISA testing.
2. ELISA based NS-1 antigen testing.

CLASSIFICATION OF SEVERE DENGUE INFECTION: (WHO CLINICAL CRITERIA, 2009):

Severe dengue infection (SDI) is defined by one or more of the following:

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

STATISTICAL ANALYSIS:

Categorical data was represented in the form of frequency. Association between variables were assessed with Chi Square Test,

Quantitative data was represented as mean & Sd. Comparison of variables has been done with ANOVA with Tukey's Multiple Comparison.

A P value of <0.05 was considered statistically significant.

Data was analyzed with IBM SPSS Version 28 for windows.

RESULTS

TABLE-1:Mean and SD of age of study subjects

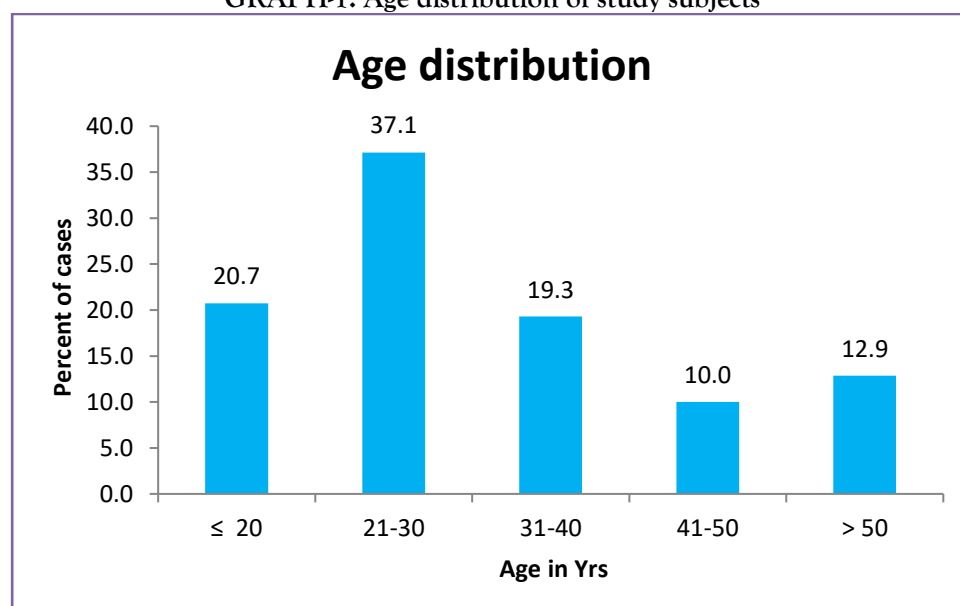
Age	
Mean	32.34
Std. Deviation	14.38
Minimum	18.00
Maximum	80.00

In our study, age of the patients ranged from 18 to 80 years with mean age of cases 32.34years and standard deviation of 14.38%

TABLE-2 Age group wise distribution of study participants

Age	No of cases	Percent
18-20	29	20.7
21-30	52	37.1
31-40	27	19.3
41-50	14	10.0
> 50	18	12.9
Total	140	100.0

GRAPH-1. Age distribution of study subjects



More than half (37.1%) of the study participants belong to the age group of 21-30years and 20.7% belong to the age group 18-20 years, and 19.3% were of 31-40 years age group. Remaining 12.9% were of more than 50 yrs and 10% were of 41-50 years age group.

TABLE-3:Gender distribution among all fever cases

Gender	No of cases	Percent
Male	108	77.1
Female	32	22.9
Total	140	100.0

From the table, male participants were predominant (77.1%) than female (22.9%)

GRAPH-2. GENDER DISTRIBUTION

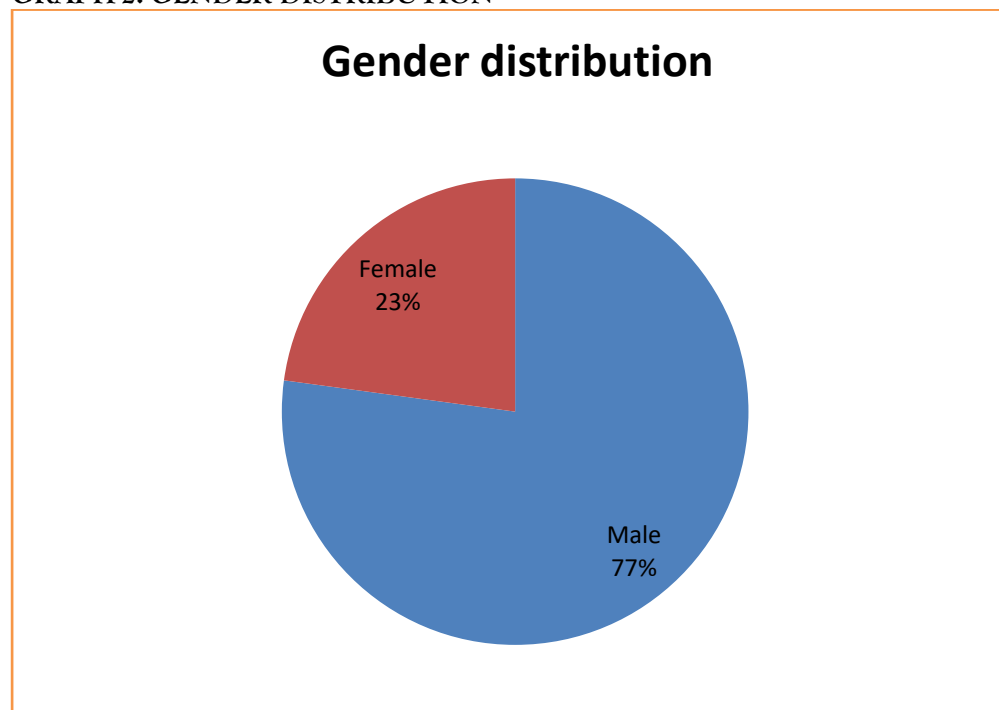
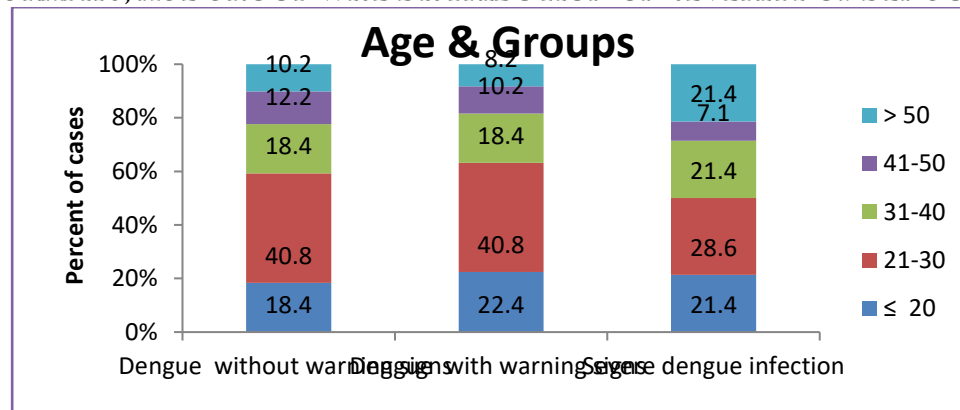


TABLE-4:Comparison between age with groups

Age		Dengue without warning signs	Dengue with warning signs	Severe Dengue Infection	Total	χ^2 Value	P Value
≤ 20	Count	9	11	9	29	5.641	0.687, Not Sig
	%	18.40%	22.40%	21.40%	0		
21-30	Count	20	20	12	52		
	%	40.80%	40.80%	28.60%	0		
31-40	Count	9	9	9	27		
	%	18.40%	18.40%	21.40%	0		

41-50	Count	6	5	3	14
	%	12.20%	10.20%	7.10%	10
> 50	Count	5	4	9	18
	%	10.20%	8.20%	21.40%	12.90%
Total	Count	49	49	42	140
	%	100%	100%	100%	100%

GRAPH-3, AGE GROUP WISE DISTRIBUTION ON SEVERITY OF DENGUE

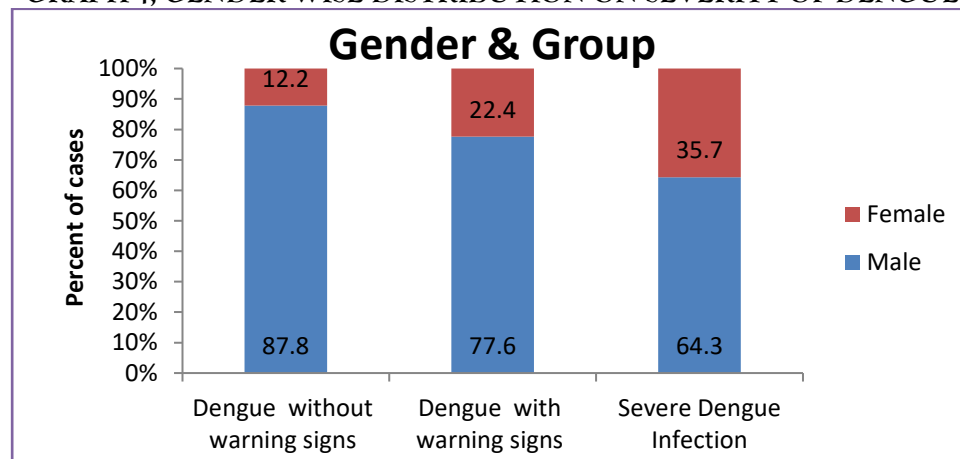


INFERENCE: There is no statistical significance between age and fever groups under study with p-value of 0.687.

TABLE-5: Comparison between gender with groups

Gender		Dengue without warning signs	Dengue with warning signs	Severe Dengue Infection	Total	χ^2 Value	P Value
Male	Count	43	38	27	108	7.072	0.029, Sig
	%	87.80%	77.60%	64.30%	77.10%		
Female	Count	6	11	15	32		
	%	12.20%	22.40%	35.70%	22.90%		
Total	Count	49	49	42	140		
	%	100%	100%	100%	100%		

GRAPH-4, GENDER WISE DISTRIBUTION ON SEVERITY OF DENGUE



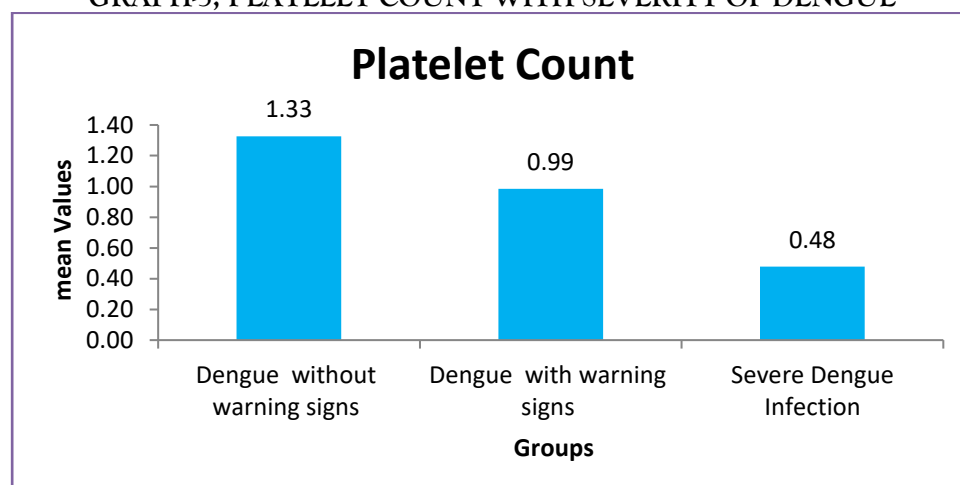
INFERENCE: There is statistical significance between gender and fever groups under study with p value

TABLE-6, COMPARISON OF PLATELET COUNT WITH DENGUE SEVERITY

Parameter	Groups	N	Mean	Std. Deviation	Minimum	Maximum	F Value	P Value
Platelet count	Dengue without warning signs	49	1.33	0.32	0.66	2.50	40.254	0.0001
	Dengue with warning signs	49	0.99	0.56	0.17	1.96		
	Severe Dengue Infection	42	0.48	0.44	0.06	2.32		
HS = Highly Sig								

Tukey Post Hoc test with Multiple Comparisons							
Dependent Variable			Mean Difference	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Platelet count	Dengue without warning signs	Dengue with warning signs	.34112 [*]	0.091	0.0001, HS	0.1254	0.5569
		Severe Dengue Infection	.84820 [*]	0.095	0.0001, HS	0.6237	1.0727
	Dengue with warning signs	Severe Dengue Infection	.50707 [*]	0.095	0.0001, HS	0.2825	0.7316
HS = Highly Sig							

GRAPH-5, PLATELET COUNT WITH SEVERITY OF DENGUE



There is high statistical significance in platelet count between dengue without warning sign and dengue with warning signs /severe dengue infection($p=0.0001$) The mean value of platelet count among patients with dengue without warning signs in 1.33lakhs/cu.mm, in patients with dengue with warning signs is 0.99lakh/cu.mm, in patients with severe dengue infection is 0.48lakh/cu.mm.

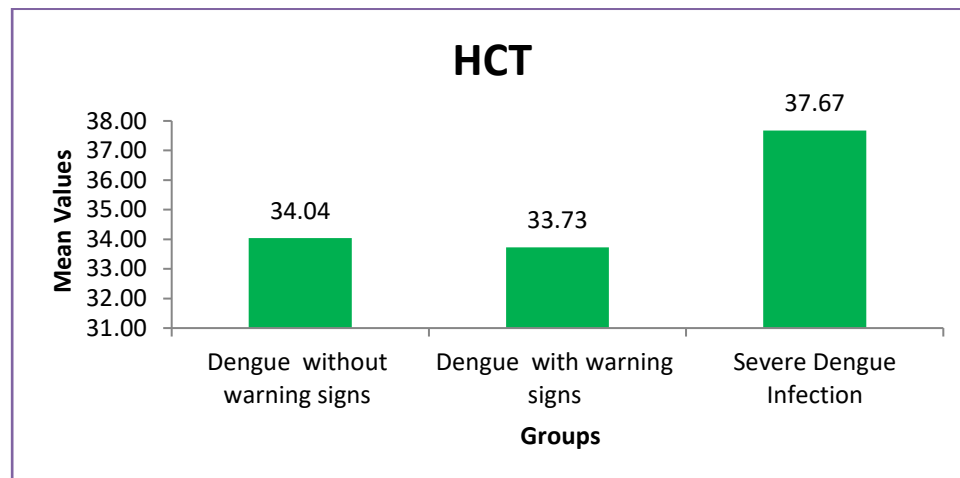
There is high statistical significance between platelet count and severity of dengue($p<0.0001$)

TABLE 7: Comparison of Hct with dengue severity

Parameter	Groups	N	Mean	Std. Deviation	Minimum	Maximum	F Value	P Value
HCT	Dengue without warning signs	49	34.04	5.16	27.00	46.00	6.645	0.002, HS
	Dengue with warning signs	49	33.73	4.98	24.00	44.50		
	Severe Dengue Infection	42	37.67	6.81	22.00	51.00		
HS = Highly Sig								

Tukey Post Hoc test with Multiple Comparisons							
Dependent Variable			Mean Difference	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
HCT	Dengue without warning signs	Dengue with warning signs	0.31224	1.141	0.959, NS	-2.3904	3.0149
		Severe Dengue Infection	-3.62891*	1.187	0.007, HS	-6.4419	-0.8159
	Dengue with warning signs	Severe Dengue Infection	-3.94116*	1.187	0.003, HS	-6.7542	-1.1282
NS = Not Sig, HS = Highly Sig							

GRAPH-6, HCT with dengue severity



There is high statistical significance in hematocrit between dengue with warning signs and severe dengue infection ($p=0.0005$)

There is no statistical significance in between hematocrit and dengue without warning signs ($p=0.959$)

The mean value of HCT in dengue without warning signs 34.04, in dengue with warning signs 33.73, and in severe dengue infection 37.67.

Table-8, COMPARISON OF SR.CAL WITHIN STUDY GROUPS

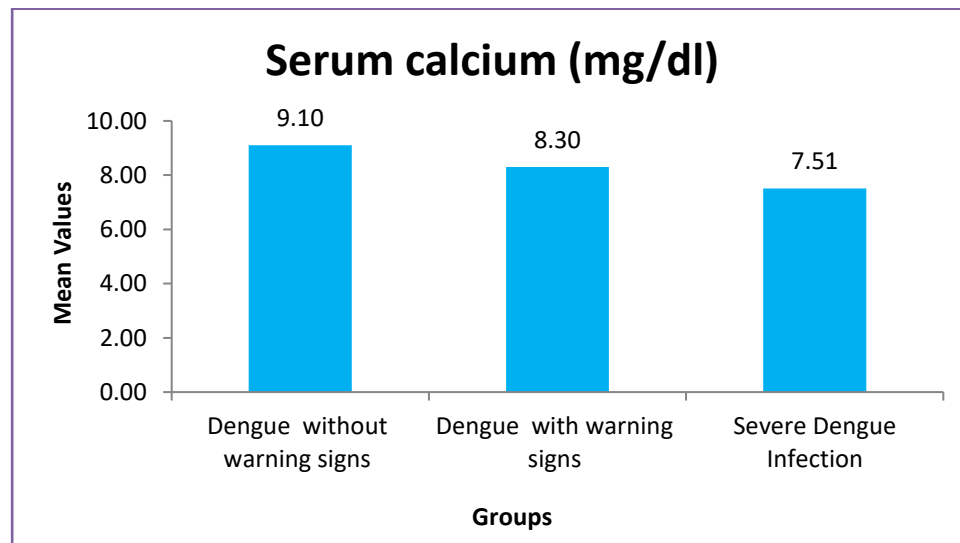
Parameter	Groups	N	Mean	Std. Deviation	Minimum	Maximum	F Value	P Value
Serum calcium (mg/dl)	Dengue without warning signs	49	9.10	0.69	7.50	10.40	43.597	0.0001, HS
	Dengue with warning signs	49	8.30	0.87	6.30	10.20		
	Severe Dengue Infection	42	7.51	0.85	5.90	9.40		

HS = Highly Sig

Tukey Post Hoc test with Multiple Comparisons							
Dependent Variable			Mean Difference	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Serum calcium (mg/dl)	Dengue without warning signs	Dengue with warning signs	.79592*	0.163	0.0001, HS	0.4094	1.1825
		Severe Dengue Infection	1.58367*	0.170	0.0001, HS	1.1813	1.9860
	Dengue with warning signs	Severe Dengue Infection	.78776*	0.170	0.0001, HS	0.3854	1.1901

HS = Highly Sig

GRAPH-7, Serum calcium with severity of dengue



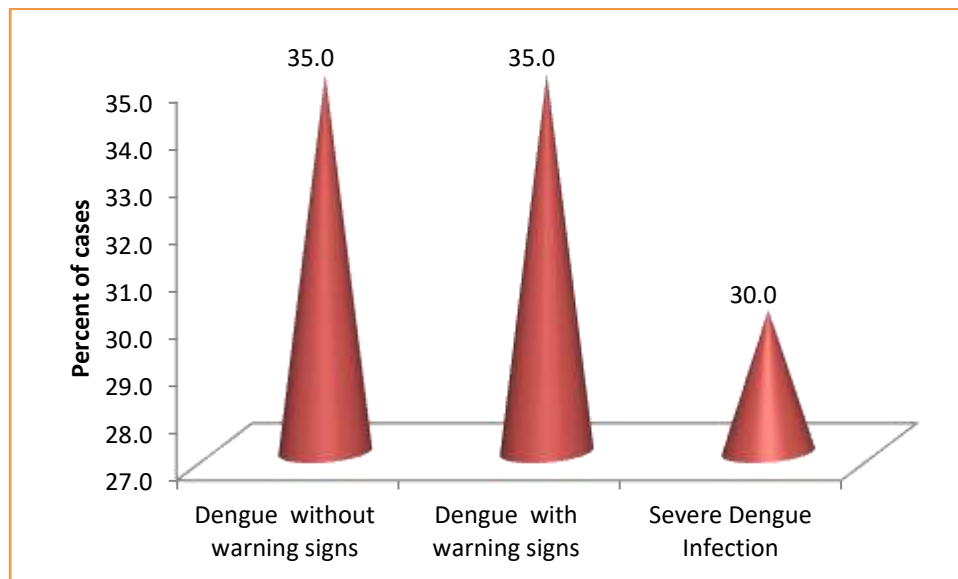
There is high statistical significance in serum calcium levels between Dengue without warning signs ($p=0.0001$), mean- 9.10 and dengue with warning signs ($p=0.0001$), mean- 8.30 and severe dengue infection ($p=0.0001$), mean-7.51

Hence, reduced serum calcium levels occurs both in dengue patient With warning signs and severe dengue infection

TABLE-9, FREQUENCY OF WARNING SIGNS AMONG DENGUE PATIENTS

Warning signs	No of cases	Percent
Dengue without warning signs	49	35.0
Dengue with warning signs	49	35.0
Severe Dengue Infection	42	30.0
Total	140	100.0

GRAPH-8, FREQUENCY OF WARNING SIGNS AMONG DENGUE PATIENTS



In our study, dengue without warning signs were 35%, Dengue with warning signs 35%, severe dengue infection 30%.

TABLE-10, VARIOUS MANIFESTATIONS OF SEVERE DENGUE

Warning signs	pleural effusion		Total
	Present	Absent	
Dengue without warning signs	1	48	49
Dengue with warning signs	4	45	49
Severe Dengue Infection	20	22	42
Total	25	115	140
Chi Square test $P < 0.001$, Highly Sig			

There is high statistical significance between pleural effusion and dengue without warning signs (case 1), dengue with warning signs (case 4), and severe dengue infection (case 20).

Warning signs	Ascites		Total
	Present	Absent	
Dengue without warning signs	0	49	49
Dengue with warning signs	4	45	49
Severe Dengue Infection	21	21	42
Total	25	115	140

Chi Square test $P < 0.001$, Highly Sig

There is high statistical significance between Ascites and dengue without warning signs (case 0), dengue with warning signs (case 4), and severe dengue infection (case 21).

Warning signs	GB wall edema		Total
	Present	Absent	
Dengue without warning signs	1	48	49
Dengue with warning signs	10	39	49
Severe Dengue Infection	26	16	42
Total	37	103	140
Chi Square test $P < 0.001$, Highly Sig			

There is high statistical significance between GB wall edema and Dengue without warning signs (case 1), dengue with warning signs (case 10), and severe dengue infection (case 26)

Warning signs	Bleeding manifestations		Total
	Present	Absent	
Dengue without warning signs	0	49	49
Dengue with warning signs	9	40	49
Severe Dengue Infection	30	12	42
Total	39	101	140
Chi Square test $P < 0.001$, Highly Sig			

There is high statistical significance between bleeding manifestations and dengue without warning signs (case 0), dengue with warning signs (case 9), and severe dengue infection. (case 30)

Warning signs	Shock		Total
	Present	Absent	
Dengue without warning signs	0	49	49
Dengue with warning signs	2	47	49
Severe Dengue Infection	9	33	42
Total	11	129	140
Chi Square test $P < 0.001$, Highly Sig			

There is high statistical significance between shock and dengue without warning signs (case 0), dengue with warning signs (cases 2), and severe dengue infection (cases 9).

TABLE 11. DURATION OF HOSPITAL STAY IN DAYS

Duration of Hospitalization (In Days)	No of cases	Percent
1-2	36	25.7
3-4	65	46.4
5-6	21	15.0
7-8	18	12.9
Total	140	100.0

More than half of the cases 21 (46.4%) were had duration of hospital stay was 3-4 days, 36 cases (25.7%) were had 1-2 days, 21 cases (15%) were had 5-6 days, 18 cases (12.9%) were had 7-8 days.

GRAPH 10. DURATION OF HOSPITAL STAY

Duration of Hospitalization (In Days)

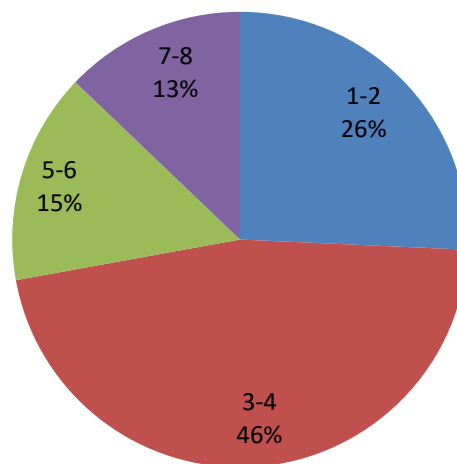
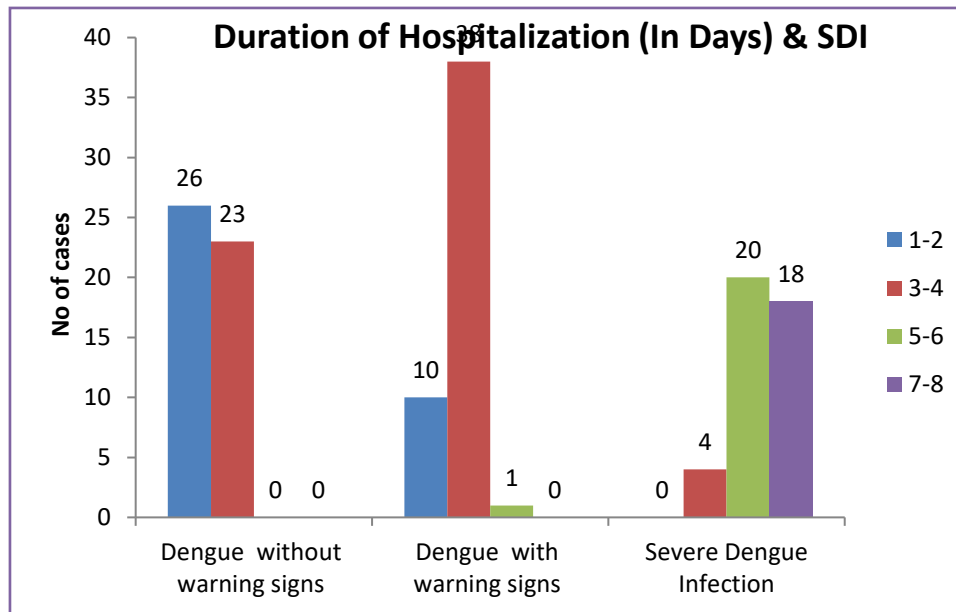


TABLE 12 COMPARISON BETWEEN DURATION OF HOSPITAL STAY AND DENGUE SEVERITY

DOH	
Mean	3.74
Std. Deviation	1.92
Minimum	1.00
Maximum	8.00

Groups	Duration of Hospitalization (In Days)			
	1-2	3-4	5-6	7-8
Dengue without warning signs	26	23	0	0
Dengue with warning signs	10	38	1	0
Severe Dengue Infection	0	4	20	18
Total	36	65	21	18
Chi Square test P<0.001, Highly Sig				

There is high statistical significance(P<0.001) between Duration of hospital stay and dengue without warning signs , dengue with warning signs, and severe dengue infection.

GRAPH 11 COMPARISON BETWEEN DURATION OF HOSPITAL STAY AND DENGUE SEVERITY

There is high statistical significance($P < 0.001$) between Duration of hospital stay and dengue without warning signs , dengue with warning signs, and severe dengue infection

Groups	N	Duration of Hospitalization (In Days)		ANOVA	
		Mean	Std. Deviation	F Value	Sig
Dengue without warning signs	49	2.39	0.76	177.83	0.001, Highly Sig
Dengue with warning signs	49	2.98	0.88		
Severe Dengue Infection	42	6.19	1.38		

There is high statistical significance($P < 0.001$) between Duration of hospital stay and dengue without warning signs(mean 2.39) , dengue with warning signs (mean 2.98), and severe dengue infection (mean 6.19).

Tukey Post Hoc test with Multiple Comparisons							
Dependent Variable			Mean Difference	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Duration of Hospitalization (In Days)	Dengue without warning signs	Dengue with warning signs	-.59184*	0.206	0.01, S	-1.0811	-0.1026

		Severe Dengue Infection	-3.80272*	0.215	0.0001, HS	-4.3119	-3.2935
	Dengue with warning signs	Severe Dengue Infection	-3.21088*	0.215	0.0001, HS	-3.7201	-2.7017
HS = Highly Sig, S = Sig							

There is high statistical significance ($P < 0.001$) between Duration of hospital stay and dengue without warning signs (mean 2.39), dengue with warning signs (mean 2.98), and severe dengue infection (mean 6.19).

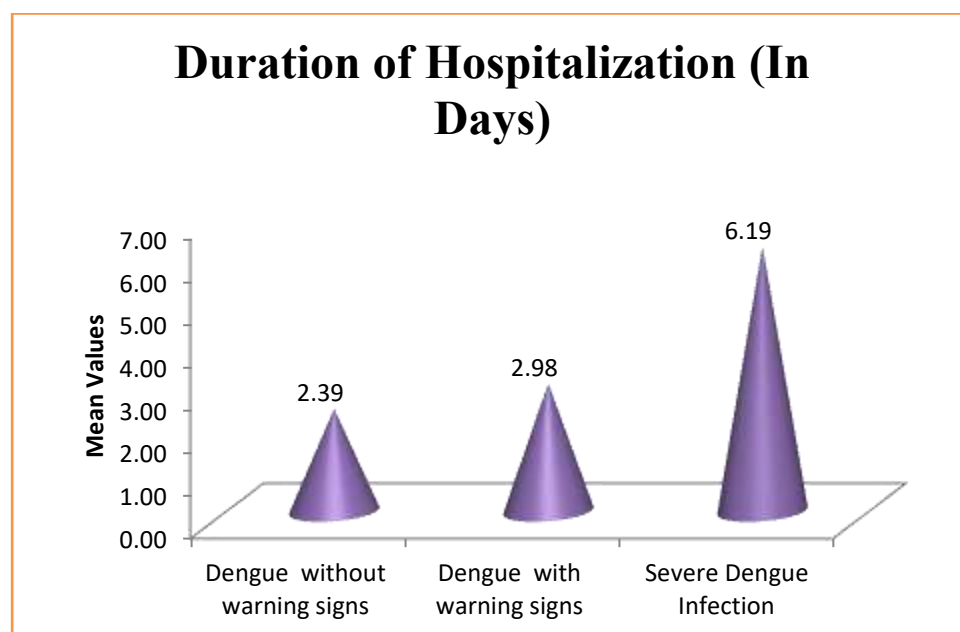


Table13. Blood transfusion and severity of dengue

Warning signs	blood transfusion		Total
	No	Yes	
Dengue without warning signs	49	0	49
Dengue with warning signs	48	1	49
Severe Dengue Infection	41	1	42

Total	138	2	140
Chi Square test $P < 0.574$, Not Sig			

There is no statistical significance ($P < 0.574$) between blood transfusion and dengue without warning signs, dengue with warning signs, and severe dengue infection.

Table 14. FFP transfusion and severity of dengue

Warning signs	FFP transfusion		Total
	No	Yes	
Dengue without warning signs	49	0	49
Dengue with warning signs	49	0	49
Severe Dengue Infection	41	1	42
Total	139	1	140
Chi Square test $P < 0.309$, Not Sig			

There is no statistical significance ($P < 0.309$) between FFP transfusion and dengue without warning signs, dengue with warning signs, and severe dengue infection.

Table 15. Platelet transfusion and Severity of dengue

Warning signs	Platelet transfusion			Total
	No	One	Two	
Dengue without warning signs	49	0	0	49
Dengue with warning signs	47	1	1	49
Severe Dengue Infection	30	8	4	42
Total	126	9	5	140
Chi Square test $P < 0.001$, Highly Sig				

There is high statistical significance ($P < 0.001$) between bleeding manifestations and dengue without warning signs, dengue with warning signs, and severe dengue infection.

6. DISCUSSION:

Dengue is a major arboviral infection spread by mosquitoes. In Southeast Asia, the Pacific, and the Americas every year, there are around 50 million dengue infections and around 500,000 individuals hospitalized with DHF. Dengue is a rapidly emerging disease in India and it has been prevalent for about 230 years here. India recorded 99913 cases and 220 deaths during a major outbreak in 2015 according to the NVBDCP.

In severe dengue infection various serum biochemical parameter changes occur due to plasma leakage. Thus analyzing the relation between serum calcium and its association with severe dengue infection may prove helpful in improving treatment outcomes. Hypocalcaemia is known to be associated with plasma leakage during severe dengue and this insists the need for studies on this areas are to improve the treatment outcomes.

In view of the above said we did a study titled "A STUDY ON CORRELATION OF SERUM CALCIUM WITH SEVERITY OF DENGUE FEVER ADMITTED AT TERTIARY CARE HOSPITAL - A CASE SERIES STUDY" in SNMC Medical college and HSK hospital Bagalakot.to assess the correlation between serum calcium as a biochemical marker of severity of dengue infection and duration of hospital stay.

Padmini Prakash Habbu et al ., at Ashwini Rural Medical College, Hospital and Research Centre, Sholapur over the period of 6month with sampling of 70 individuals studied Hb, SGOT and SGPT, creatinine and calcium estimations of Healthy control and Dengue patients and found that in dengue patients Calcium level decreased in DF range from 5.5-10 mg/dl and 8-11 mg/dl among the controls the Hb values are low as compared to Healthy controls it's ranging from 6.0 - 11gm/dl in dengue patients and 10.0-15.0gm/dl in Healthy controls. SGOT and SGPT values are raised in dengue as compared to healthy controls it's ranging between 56 - 532 IU/L among dengue patients and 19-60IU/L among healthy control groups. The creatinine values are almost same in control and patients ranging between 0.5- 2.8 mg/dl. Calcium level decreased in DF range from 5.5-10mg/dl and 8-11mg/dl among the controls[37]

Jayachandra et al., of Department of Medicine, BMC, Bangalore conducted a study using 145 patients and concluded that hypocalcemia is associated with severe dengue infection compared with dengue fever patients without warning signs.[38]

Mitrakrishnan C Shivanthan et al .,of University of Colombo, Department of Medicine, Sri Lanka studied the relation between dengue infection and serum calcium levels and found that dengue related Myocarditis has a relationship with alteration in intracellular calcium level. They also observed that there is increased mortality in severe dengue with hypocalcemia.[39]

N J Dahanayaka et al.,studied the significance of detecting hypocalcaemia to predict severity of dengue infection .Using 36 probable cases of dengue conducted across sectional study at University Medical Unit (UMU), Teaching Hospital Anuradhapura and found that Positive and negative predictive values of hypocalcaemia predicting TSFA was 29% and 100% respectively with a positive likelihood ratio of 2.17 (95% CI 1.84-2.551). Mean SIC(lowest) among patients with TSFA(Third space fluid accumulation) was 0.97 (SD 0.1) mmol/l compared to 1.12 (SD 0.14) mmol/l among those without TSFA (p=0.035). Patients with hypocalcaemia had significantly lower platelet count and serum albumin levels (Table3). All five patients with platelet count $<20000 \times 10^9/L$ had hypocalcemia.[40]

Constantine GR et al., Studied the association of Hypocalcemia with disease severity using 135 patients in SriLanka and found that there is significant correlation between dengue severity and SerumCa²⁺levels.[41]

Madura Adikari et al ., of Colombo North Teaching Hospital, Ragama,Sri Lanka conducted a prospective follow up studyin a tertiary care centre in Sri Lanka throughout a one year period in 61 patients with severe dengue infection and found that 52(85%) showed hypocalcemia during the first 24 hours of onset of severe dengue infection. Mean ionized calcium level of the population was 0.96mmol/L and the range being 0.53-1.48mmol/L. There was major reduction in serum calcium level within the first 24 hours of the onset of severe dengue clinical criteria.[42]

Dr. Aditya Mahajan et al ., of Department Of General Medicine, A J Institute Of Medical Sciences / Rajeev Gandhi University Of Health Sciences, India conducted a study over 2 years on Correlation between Serum Ionized Calcium and Severity of Dengue Infection using across -sectional study done at A.J Institute of Medical Sciences in Mangalore, Karnataka with 50 probable cases and found that a statistically significant association was found between serum ionized calcium and dengue severity(p value- <0.000001) . It was also observed that there was a statistically significant association (p value- <0.000001)

between serum ionized calcium and hematocrit (p value- <0.000001) Also a statistically significant association was found between serum ionized calcium and ALT levels (pvalue-<0.000001).[43]

Alagarasu K et al., done a study in Maharashtra comparing the Vitamin D levels in dengue infection with those that of healthy population and concluded that it was significantly high in dengue patients.[44]

7. LIMITATIONS:

- The time of measurement in serum calcium level is not constant in all patients because, it was measured at different times of illness in various patients. Hence, we cannot conclude that at which stage of disease calcium level started to decline.
- Study population includes age group of only 18-80 yrs. Beyond this range of age the pattern of correlation between calcium and dengue could not be concluded.
- The duration of hypocalcemia in dengue is not constant in all patients and hence onset, duration and recovery from hypocalcemia could not be defined.
- The present study was done in a limited number of subjects, the results may vary if done in a large number of subjects.
- It is a single center study.
- Being a hospital-based study, there is bias factor in selection of subjects. Only symptomatic patients who presented to hospital were studied and the study population might not be representative of the population of Bagalkot.

8. CONCLUSION:

A total of 140 cases were studied.

Dengue diagnosis was made by detection of NS-1 antigen and IgM antibody.

All cases were seropositive for dengue (100%)

Dengue cases were classified according to WHO guidelines. Dengue positive cases (NS-1/IgM) was split into 3 category

- Dengue fever (DF-49 cases (35%))
- Dengue fever with warning signs-49 cases(35%)
- Severe dengue infection-42 cases(30%)

In severe dengue infection (total of 42 cases), 9 (21.4%) cases presented with shock, Patients presented with pleural effusion 20 (47.61%), ascites 21 (50%) and gall bladder wall edema 26(61.9%), 30 (71.42%) cases presented with various bleeding manifestations. Platelet count and rise of hematocrit in dengue fever was significantly correlated with disease severity.

There is high statistical significance (p=0.0001) in serum calcium level between dengue without warning signs (Mean serum calcium level=9.10meq/l) and dengue with warning signs (Mean serum calcium level=8.30meq/l) / severe dengue(Mean serum calcium level =7.51meq/l).

Hence, incidence of hypocalcemia occurs more frequently in dengue cases with warning signs and severe dengue infection.

There is high statistical significance (P<0.001) in duration of hospital stay between dengue without warning signs (mean duration of hospital stay 2.39 days), dengue with warning signs (mean duration of hospital stay 2.98 days) and severe dengue infection (mean duration of hospital stay 6.19 days).

Hence the increased duration of hospital stay correlate with severity of dengue and severity of hypocalcemia.

Hence, it was observed that serum calcium levels shows significant correlation with dengue fever severity and duration of hospital stay. The Mean serum calcium levels was significantly lower in cases with severe dengue infection and dengue fever with warning signs than in patients with dengue fever without warning signs. So the increased duration of hospital stay correlate with severity of dengue and severity of hypocalcemia.

Furthermore, the serum calcium levels can be used as a potential bio-marker to predict the severity of dengue infection and can be used a prognostic marker as well. But further studies are needed to

support this.

We suggest further studies in the following areas i) Role of calcium therapy as a part of dengue infection treatment ii) effects of hypocalcemia on cardiac and skeletal muscles in dengue fever.

BIBLIOGRAPHY:

- World Health Organization and Tropical Diseases Research. Dengue: Guidelines for diagnosis, treatment, prevention and control. Geneva: World Health Organization; 2009: new edition.
- Dengue: Guidelines for treatment, prevention and control. Geneva: World Health Organization, 2009.
- Baruah K, Biswas A, Suneesh K, Dhariwal AC. Dengue fever: Epidemiology and clinical pathogenesis. Chapter 13, Major tropical diseases: Public health perspective. Goa: Broadway publishing House; 2014:255-71.
- Dash AP, Bhatia R, Kalra NL. Dengue in South East Asia: An appraisal of case management and vector control. Dengue Bulletin. 2012;36:1-12.
- Kalaya narooj S. Clinical manifestations and management of dengue/DHF/DSS. Tropical Medicine and Health. 2011;39(4suppl):83-9.
- National Guidelines for Clinical Management of dengue Fever Prof (Dr) Jagdish Prasad, Director General of Health Services, Government of India released the new Guidelines with Special Focus on Case Management
- Simmons, C., Farrar, J., van Vinh Chau, N. and Wills, B. (2012). Dengue. New England Journal of Medicine, 366(15), pp.1423-1432.
- Wali J, Biswas A, Aggarwal P, Wig N, Handa R. Validity of tourniquet test in dengue hemorrhagic fever, J Assoc Physicians India. 1999;47(2):203-204.
- Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever. Revised and expanded edition. (SEARO Technical Publication Series No. 60 ISBN 978-92-9022-387-0)
- Peeling RW, Artsob H, Pelegriño JL, et al. Evaluation of diagnostic tests: dengue. Nat Rev Microbiol 2010;8:Suppl:S30-S38.
- Zaloga GP. Hypocalcemia in critically ill patients. Crit Care Med. 1992;20:251-62.
- Zivin JR, Gooley T, Zager RA, Ryan MJ. Hypocalcemia: a pervasive metabolic abnormality in the critically ill. Am J Kidney Dis. 2001; 37:689-98.
- Vivien B, Langeron O, Morell E, Devilliers C, Carli P A, Coriat P, Riou B. Early hypocalcemia in severe trauma. Crit Care Med. 2005;33:1946-52.
- Carlstedt F, Lind L, Rastad J, Stjernström H, Wide L, Ljunghall S. Parathyroid hormone and ionized calcium levels are related to the severity of illness and survival in critically ill patients. Eur J Clin Invest. 1998;28:898-903.
- Burchard KW, Gann DS, Colliton J, Forster J. Ionized calcium, parathormone, and mortality in critically ill surgical patients. Ann Surg. 1990;212:543-50.
- Forman DT, Lorenzo L. Ionized calcium: its significance and clinical usefulness. Ann Clin Lab Sci. 1991;21:297-304.
- Zaloga GP. Hypocalcemia in critically ill patients. Crit Care Med. 1992;20:251-62.
- 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.
- Cabrera-Cortina JI, Sanchez-Valdez E, Cedas-DeLezama D, Ramirez-Gonzalez MD (2008) Oral calcium administration attenuates thrombocytopenia in patients with dengue fever. Report of a pilot study. P W Pharmacol Soc 51:38-41.
- Singh PS, Singh N (2012) Tetany with Plasmodium falciparum infection. J Assoc Physicians India 60:57-58.
- Desai T K, Carlson R W, Geheb M A (1988) Prevalence and clinical implications of hypocalcemia in acutely ill patients in a medical intensive care setting. Am J Med 84:209-214.
- Baines P B, Thomson A P, Fraser W D, Hart CA (2000) Hypocalcaemia in severe meningococcal infections. Arch Dis Child 83:510-513.
- Khanna M, Chaturvedi UC, Dhawan R, Tekwani BL, Pandey VC. Presence of Ca²⁺ is obligatory for the cytotoxic activity of dengue virus-induced cytotoxic factor. Immunology 1991;72:73-8.
- Dhawan R, Chaturvedi UC, Khanna M, Mathur A, Tekwani BL, Pandey VC, Rai R N. Obligatory role of Ca²⁺ in the cytotoxic activity of dengue virus-induced cytotoxin. Int J Exp Pathol 1991;72:31-9.
- Cabrera-Cortina J I, Sánchez-Valdéz E, Cedás-DeLezama D, Ramírez-González MD. Oral calcium administration attenuates thrombocytopenia in patients with dengue fever. Report of a pilot study. Proc West Pharmacol Soc 2008;51:38-41.
- Sitprija V. Altered fluid, electrolyte and mineral status in tropical disease, with an emphasis on malaria and leptospirosis. Nat Clin Pract Nephrol. 2008;4:91-101. [PubMed]
- Bunnag T, Kalayanarooj S. J Med Assoc Thai. Vol. 94. Bangkok, Thailand: 2011. Dengue shock syndrome at the emergency room of Queen Sirikit National Institute of Child Health; pp. S57-63. [PubMed]
- Zaloga GP, Chernow B. The multifactorial basis for hypocalcemia during sepsis. Studies of the parathyroid hormone-vitamin D axis. Ann Intern Med. 1987;107:3641. [PubMed]
- Uddin KN, Musa AKM, Haque WMM, Sarker RSC, Ahmed AKMS. A follow up on biochemical parameters in dengue patients attending BIRDEM hospital. Ibrahim Med Coll J. 2008;2:25-7.
- Wiwanitkit S, Wiwanitkit V. Hypocalcemia, tetany and dengue. Indian J Pediatr. 2012;80:618. [PubMed]
- Khare M, Chaturvedi U C. Transmission of dengue virus-specific suppressor signal depends on the presence of calcium. Indian

- J Med Res.1995;102:1-8.[PubMed]
- Misra A, Mukerjee R, Chaturvedi UC. Production of nitrite by dengue virus-induced cytotoxic factor. Clin Exp Immunol. 1996;104:406-11.[PMCFreearticle][PubMed]
 - Mukerjee R, Misra A, Chaturvedi UC. Dengue virus-induced cytotoxin releases nitrite by spleen cells. Int J Exp Pathol. 1996;77:45-51. [PMCFreearticle][PubMed]
 - Wichmann D, Kularatne S, Ehrhardt S, Wijesinghe S, Brattig NW, Abel W, et al. Cardiac involvement in dengue virus infections during the 2004/2005 dengue fever season in Sri Lanka. Southeast Asian J Trop Med Public Health. 2009;40:727-30.[PubMed]
 - Kularatne SA, Pathirage MM, Kumarasiri PV, Gunasena S, Mahindawansa SI. Cardiac complications of a dengue fever outbreak in Sri Lanka, 2005. Trans R Soc Trop Med Hyg. 2007;101:804-8.[PubMed]
 - Salgado DM, Eltit JM, Mansfield K, Panqueba C, Castro D, Vega MR, et al. Heart and skeletal muscle are targets of dengue virus infection. Pediatr Infect Dis J. 2010;29:238-42.[PMCFreearticle][PubMed]
 - Habbu PP, Shaikh AK. Dengue fever: an observational study in area of Solapur, Maharashtra. Int J Health Sci Res. 2015;5(3):169-172.
 - Jayachandran I, Kavya S T1, Sphoori P Pai1, Balakrishna A. Utility of serum free calcium as a predictor of severity in dengue fever. Bangalore Medical College and Research Institute, Department of Medicine, Bangalore. Cukurova Medical Journal 2017;42(4):609-616
 - Shivanthan, Mitrakrishnan C, and Senaka Rajapakse. "Dengue and calcium." International journal of critical illness and injury science vol.4,4(2014):314-6. doi:10.4103/2229-5151.147538
 - Dahanayaka, N.J., Agampodi, S.B., Kodithuwakku Arachchi, U.P.K.A., Vithange, S.P., Rajapakse, R., Ranathunga, K. and Siribaddana, S., 2017. Dengue fever and ionized calcium levels: significance of detecting hypocalcaemia to predict severity of dengue. Ceylon Medical Journal, 62(1), pp.67-69. DOI: <http://doi.org/10.4038/cmj.v62i1.8438>
 - Constantine G, Rajapakse S, Ranasinghe P, Parthippan B, Wijewickrama A, Jayawardana P (2014) Hypocalcemia is associated with disease severity in patients with dengue. J Infect Dev Ctries 8:1205-1209. doi:10.3855/jidc.4974
 - Adikari M, Perera C, Fernando M, Loeb M, Premawansa S, et al. (2015) Prevalence of Hypocalcemia and Its Potential Value as a Biochemical Marker in Patients with Severe Dengue Infection. J Trop Dis 4: 188. doi:10.4172/2329-891X.1000188
 - Dr. Aditya Mahajan. "A Study on Correlation between Serum Ionized Calcium and Severity of Dengue Infection." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol.18, no.3, 2019, pp.44-52.
 - Alagarasu K, Bachal RV, Bhagat AB, Shah PS, Dayaraj C. Elevated levels of vitamin D and deficiency of mannose binding lectin in dengue hemorrhagic fever. Virol J. 2012;9:86.

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ANNEXURE

CONSENT FORM

Name of the study: **A STUDY ON CORRELATION OF SERUM CALCIUM WITH SEVERITY OF DENGUE FEVER ADMITTED AT TERTIARY CARE HOSPITAL - A CASE SERIES STUDY.**

I / We..... hereby have been explained and understand that during the antenatal visits at this hospital, certain investigations are required for the purpose of diagnosis/treatment.

I/ we understand that the research is designed to study Urinary tract infection in pregnant women and the organisms which are responsible for the infection. We understand that these reports have to be stored in medical records department for future reference.

I / we hereby voluntarily agree and accept to give our consent for preservation of these materials and also that these materials/data/information or reports obtained from them may be utilized for research purpose in future for the benefit of other mankind/population.

I/We understand that strict confidentiality will be maintained and also that our refusal to give consent will in no way interfere in the treatment process.