A cross sectional study of association between severity of hypocalcaemia and Dengue subgroup among patients in a tertiary care hospital in South Gujarat

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How to citation this article: Dr. Rajiv Pandya, Dr. Chirag Patel, Dr. Mahesh Solu, "A cross sectional study of association between severity of hypocalcaemia and Dengue subgroup among patients in a tertiary care hospital in South Gujarat", IJMACR- May - June - 2022, Vol – 5, Issue - 3, P. No. 317 – 323.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Introduction: Dengue is a disease of major concern through the world due to its ability to cause huge burden on public health system since it is rapidly transmitted by mosquito. Dengue fever is a mosquito born tropical disease caused by the Dengue virus. Serum calcium level is known to be important in cardiac and circulatory function.

Methodology: A cross sectional study of association between hypocalcaemia and its correlation with severity in Dengue fever patients in a tertiary care hospital in south Gujarat in 210 patients of Dengue NS1 and IGM positive infections according to WHO criteria.

Result: The sample size was 210. The mean age was 34.61 years. DF was diagnosed in 114 patients(54.3 %) and DHF was diagnosed in 67 patients (31.9%) and DSS was diagnosed in 29 patients(13.8%).In our study levels of serum calcium were significantly lower in DSS as compared to DHF and DF. The mean value of serum calcium was 9.46 ± 0.47 in DF, 8.40 ± 0.65 in DHF and 7.41 ± 1.07 in DSS. Serum calcium was significantly associated with DF, DHF and DSS at $p < 0.01$. As Dengue severity increased level of serum calcium was decreased.

Conclusion: It was observed that serum calcium levels shows significant correlation with Dengue fever severity. 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. 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.

Keywords: Dengue, Dengue Hemorrhagic fever, Serum calcium,

Introduction

Dengue is a disease of major concern through the world due to its ability to cause huge burden on public health

system since it is rapidly transmitted by mosquito. Dengue fever is a mosquito-borne tropical disease caused by the Dengue virus. Symptoms typically begin three to fourteen days after infection.

These may include a high fever, headache, vomiting, muscle and joint pain, and a characteristic skin rash.

Recovery generally takes two to seven days. In a small proportion of cases, the disease develops into severe Dengue, also known as Dengue hemorrhagic fever, resulting in bleeding, low levels of blood platelets and blood plasma leakage or into Dengue shock syndrome, where dangerously low blood pressure occurs (1).

The *Aedes aegypti* mosquito is the main vector that transmits the viruses that cause Dengue. The viruses are passed on to humans through the bites of an infective female *Aedes* mosquito, which mainly acquires the virus while feeding on the blood of an infected person.

Once infected, humans become the main carriers and multipliers of the virus, serving as a source of the virus for uninfected mosquitoes. The virus circulates in the blood of an infected person for 2-7 days, at approximately the same time that the person develops a fever. Patients who are already infected with the Dengue virus can transmit the infection via *Aedes* mosquitoes after the first symptoms appear (during 4-5 days; maximum 12 days). (2)

The primary method of controlling *A.aegypti* is by eliminating its habitats. This is done by getting rid of open source of water or if possible by adding insecticides or biological control agents to these areas. Reducing open collections of water through environmental modification is the preferred method of control, given the concerns of negative health effects from insecticides and greater logistical difficulties with control agents. People can prevent mosquito bites by wearing clothing that fully covers the skin, using mosquito netting while resting, and the application of insect repellent (DEET being the most effective) (3).

In India, particularly in Gujarat state in recent years, Dengue has been a major health issue contributing to significant mortality and morbidity. The major factors contributing to this mortality is severe form of Dengue infection and its complications like shock syndrome, hemorrhagic manifestations and severe thrombocytopenia. So we need to identify the patients who are all going to these complications.

In patients with severe Dengue infection numerous serum biochemical parameter changes occur with the onset of plasma leakage, these derangements are not apparent in non-severe Dengue patients. The various biochemical markers has been measured to identify the severe form of Dengue infection like AST, ALT, platelet count, PCV and electrolytes especially calcium levels.

Material And Method

Study design: Cross sectional study

Study setting: Department of General Medicine, Tertiary care hospital, Surat.

Study Participants: All patients of Dengue fever who were attending the Tertiary care hospital, Surat to avail the health services.

Inclusion criteria

- Patients over 18 years old with Dengue IgM or NS1 Antigen positive
- Admitted patients who give consent for the stud
  Exclusion criteria
- Patient who get calcium oral or intravenous before affirmation
- Pregnant women
- Patients with cirrhosis of liver

- Patient determined to have Malaria, Enteric fever and other infection
- Patient who are diagnosed with disease in which calcium level is altered (hyperparathyroidism, chronic kidney failure, pancreatitis, tumor lysis syndrome)

Sample size calculation:

All patients determined to have Dengue by NS1/IgM during the information assortment period were remembered for this review following incorporation and rejection rules

Sample size: 210

Sampling method: Purposive testing technique was taken on to choose the study participants.

Study tool: Pre tested, pre designed, semi structured questionnaire

Procedure

All patients' medical history were taken and general physical examination was done. Ultrasonography (Abdomen) was done. A blood sample from each patient was collected to measure:

➢ Dengue IgM Antibody/ Dengue NS1 Antigen

➢ Complete blood count

➢ Serum calcium level

➢ Liver capacity test (Serum Alanine Aminotransferase, Aspartate Aminotransferase, Total Bilirubin, Direct Bilirubin, Indirect Bilirubin)

Specimen collection: Non-hemolysed serum and plasma were used.

Radiological investigations: USG-Abdomen: To find out third space fluid collection. (Such as ascites, pleural effusion, Gall bladder wall edema)

Chest X Ray: To find out pleural effusion.

Estimation of calcium

Method: Arsenaz

Mode: Endpoin

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

1. Calcium standard: 10mg/dl
2. Arsenazo III
3. Buffe

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 650 nm

Calculation of serum calcium

Serum Calcium (mg/dl) $=(Abs.T)-(Abs.B)*10/(Abs.S)-(Abs.B)*$

Reference Range: Serum calcium 8.5 - 10.5 mg/dl

Linearity: The kit is linear up to 16 mg/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)

Serious Dengue contamination is characterized by at least one of the accompanying

➢ Plasma spillage that might prompt shock (Dengue shock) and additionally liquid collection, with or without respiratory trouble, as well as

➢ Severe bleeding, and/or

➢ Severe organ impairment, of which most occur during the critical phase.

Result

Table 1: Diagnosis of Dengue infection

Diagnosis	Cases	Percentage (%)
Dengue fever (DF)	114	54.3
Dengue Hemorrhagic Fever (DHF)	67	31.9
Dengue Shock Syndrome (DSS)	29	13.8

As per WHO classification, out of 210 patients, 54.3% were classified as Dengue Fever, 31.9% as Dengue Hemorrhagic Fever and 13.8% as Dengue Shock Syndrome. Mean age of the participants was 34.61 ± 11.49 years.

Table 2: Distribution of participants according to symptomatology

Symptoms	Number	Percentage (%)
Fever	210	100.0
Headache	161	76.7
Retro orbital pain	21	10.0
Myalgia	28	13.3
Arthralgia	38	18.1
Rash	55	26.2
Hemorrhagic manifestation	86	41.0
Nausea/Vomiting	84	40.0
Abdominal pain	41	19.5
Sleep reduced	19	9.0
Appetite decreased	36	17.1
Urine output decreased (per min)	27	12.9
Pallor	6	2.9
Icterus	14	6.7
Cyanosis	4	1.9
Clubbing	3	1.4
Lymphadenopathy	1	0.5
Edema	14	6.7

More than three fourth (76.7%) of the patients had experienced Headache and it was commonest symptom. It was followed by haemorrhagic manifestation (41.0%) and Nausea/Vomiting (40.0%)

Table 3: Association between Symptomatology and Dengue subgroups

Parameters	Dengue fever		Dengue Haemorrhagic Fever		Dengue Shock Syndrome		p-value (Chi Square Test)
	n = 114	%	n = 67	%	n = 29	%	
Fever	114	100.0	67	100.0	29	100.0	**
Hemorrhagic manifestation	0	0	61	91.04	25	86.21	<0.01
Headache	87	76.32	48	71.64	26	89.66	0.16
Arthralgia	7	6.14	13	19.40	18	62.07	<0.01
Rash	5	4.38	24	35.82	26	89.66	<0.01
Nausea/Vomiting	23	20.18	34	50.74	27	93.10	<0.01
Abdominal pain	17	14.91	14	20.90	10	34.48	0.06
Retro orbital pain	12	10.53	5	7.46	4	13.79	0.61
Myalgia	16	14.03	7	10.45	5	17.24	0.63

Fever was present in all of the Dengue subgroups. Occurrence of, Hemorrhagic Manifestation, Arthralgia, Rash and Nausea-Vomiting were statistically significantly higher in DHF and DSS as compared to classical Dengue fever patients.

**No statistics were calculated for fever as the findings were constant in all subgroups of Dengue.

Table 4: Association between serum calcium and Dengue subgroups

Parameters	Dengue Fever	Dengue Haemorrhagic Fever	Dengue Shock Syndrome	p-value (ANOVA Test)
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	
S. Calcium	9.46 $\pm$ 0.47	8.40 $\pm$ 0.65	7.41 $\pm$ 1.07	< 0.01

Serum calcium was significantly associated with DF, DHF and DSS at $p < 0.01$. As Dengue severity increased level of serum calcium was decreased.

Discussion

Dengue is a major arboviral infection spread by mosquitoes Dengue is a rapidly emerging disease in India and it has been prevalent for about 230 years here. In severe Dengue infection various serum biochemical parameter changes occur due to plasma leakage. Thus analyzing the relation between serum free 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 area so as to improve the treatment outcomes.

Symptomatology in Dengue subgroups

In our study fever was present in all subgroups of Dengue fever. Headache, retroorbital pain and myalgia were present in all subgroups of Dengue fever. Occurrence of Arthralgia, Rash, Haemorrhagic Manifestation, Nausea-Vomiting, and abdominal pain were statistically significant in DHF and DSS as compared to classical Dengue fever patients. Also the

findings of icterus [27.59%] was significantly associated with DSS in our study. Jaundice in Dengue infection has been associated with fulminant liver failure and by itself is a poor prognostic factor. In the study conducted by Murlidhar et al in 2016, fever and headache were present in all subgroups of Dengue fever. Occurrence of Myalgia, Retroorbital pain, Arthralgia, Rash, Haemorrhagic Manifestation, Nausea-Vomiting, and abdominal pain were statistically significant in DHF and DSS as compared to classical Dengue fever patients. In this study also the finding of icterus was significant in DSS [100%] as compared to DF and DHF (4).

In the study conducted by Jaykumar et al in 2012, Fever, Headache, retroorbital pain were present in all subgroups of Dengue fever. Occurrence of Arthralgia, Myalgia,Rash, Haemorrhagic Manifestation, Nausea-Vomiting, and abdominal pain were statistically significant in DHF and DSS as compared to classical Dengue fever patients (5).

In the study conducted by Daniel et al 2003, 62.4% patients complained of abdominal pain as compared to 19.5% in our study. Headache was present in 77.2% patients similar to our study [76.7%].13.2% patients had rash compared to 26.2% in our study predominantly in DHF and DSS subgroups.15.2% patients had bleeding manifestations compared to 41.0% in our study predominantly in DHF and DSS subgroups (6).

In the study conducted by Rajoo et al in 2006, fever was present in all subgroups of Dengue fever[100%],40% patients had bleeding manifestations compared to 41% in our study predominantly in DHF and DSS subgroups, Myalgia was present in only 43% compared to 13.3% in our study. 20 % patients complained of abdominal pain as compared to 19.5% in our study. (7).

Serum calcium level in Dengue subgroup

In our study levels of serum calcium were significantly lower in DSS as compared to DHF and DF. The mean value of serum calcium was 9.46 ± 0.47 in DF, 8.40 ± 0.65 in DHF and 7.41 ± 1.07 in DSS. Serum calcium was significantly associated with DF, DHF and DSS at $p < 0.01$. As Dengue severity increased level of serum calcium was decreased.

Padmini Prakash Habbu et al, at Ashwini Rural Medical College, Hospital and Research Centre, Sholapur over the period of 6 month with sampling of 70 individuals studied 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. (8).

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 (9).

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 there is increased mortality in severe Dengue with hypocalcemia (10).

Constantine GR et al., Studied the association of Hypocalcemia with disease severity using 135 patients in Sri Lanka and found that there is significant correlation between Dengue severity and Serum Ca2+ levels (11).

Conclusion

It was observed that serum calcium levels shows significant correlation with Dengue fever severity. 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. 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.

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