

Study on Association of Vitamin D Deficiency and Severity of Symptoms in Dengue Patients Admitted in A Tertiary Care Centre in North India: A Cross-Sectional Study

Jyoti Verma¹, Jyoti Pankaj², Ankit Singh³

¹Associate Professor, Department of Medicine, Dr. Ram Manohar Lohia Institute of Medical Sciences, UP

²Assistant Professor, Department of Medicine, Dr. Ram Manohar Lohia Institute of Medical Sciences, UP

³Senior Resident, Department of Medicine, Dr. Ram Manohar Lohia Institute of Medical Sciences, UP

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Corresponding author: Dr. Jyoti Verma

Conflict of interest: Nil

Abstract:

Introduction: The severity of symptoms of Dengue patients depends upon their nutritional status. Therefore, study on relation of vitamin D deficiency with severity of dengue patients needed attention. Vitamin D plays a crucial role in regulating bone metabolism and maintaining calcium and phosphorus levels in the body. It has been found to be associated with autoimmune diseases, metabolic syndromes, cardiovascular disease, cancers, and all-cause mortality. There is an increasing interest in the potential role of vitamin D in Dengue viral infection. Dengue patients had major complain of severe bodyache, fatigue when other symptoms are absent, which is variable in different patients.

Aim: To identify a possible association between low levels of systemic 25-(OH) D and the likelihood of developing severe dengue fever.

Materials and Methods: This was a single-center, cross-sectional study conducted in the Department of Medicine, Dr. RMLIMS, Lucknow, Total 156 Dengue patients were taken after confirmation from NS1 Ag and IgM positive reports. Serum vitamin D levels were measured at the time of admission in all patients. MS Excel and R-4.3.2 statistical software were used for statistical analysis. A p-value < 0.05 was considered significant. All statistical tests were two-sided.

Results: The results reflected mean age of 36 years with SD of 14.428 and low Serum Vitamin D < 20 ng/ml (P-value 0.944). Out of total (n= 156), 47.4% (n=74) were females and 52.6% (n=82) were males. The levels of vitamin D ranges between 5 and 109 ng/ml. Serum vitamin D was found deficient in 66.7% (n=102) of total Dengue patients. The prevalence of Dengue without warning signs, Dengue with warning signs and severe Dengue fever (according to the latest classification of WHO- 2021, for Dengue) was found to be 69.5%, 75% and 89% respectively in low vitamin D patients. Studies were also done for various demographic characteristics and clinical features in dengue patients with vitamin D deficiency.

Conclusion: The study concludes that severity of Dengue patients was directly related and more in low vitamin D patients. The present study revealed that serum vitamin D may contribute in the pathogenesis of dengue infection; thus, vitamin D levels might serve as useful prognostic marker for predicting disease progression.

Keywords: Serum 25-(OH) D, Dengue Fever, Bone Metabolism, Autoimmune Diseases, Metabolic Syndromes.

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Introduction

Dengue is a serious febrile illness which is caused by flaviviruses that are transmitted through mosquito bites. It is common in tropical and subtropical regions, especially in Southeast Asia, Latin America, the Caribbean and the Pacific Islands [1]. There are four different types of Dengue virus (DENV1, DENV2, DENV3 and DENV4) [1]. The first infection with any of these types is usually mild or asymptomatic. However if a person gets infected with a different type of dengue virus after the first infection, they may

experience severe dengue symptoms. It is estimated that around 50 million people are affected by dengue fever each year in countries where dengue is common, and about 70% of these cases are reported in Asia.[1]The symptoms of dengue fever can range from no symptoms at all to a variety of symptoms. Dengue fever can cause a mild flu like syndrome or a more severe form of the disease known as Dengue Hemorrhagic fever. Dengue hemorrhagic fever is characterised by bleeding, a significant decrease in the number of platelets, and

an increase in vascular permeability that can cause hemo-concentration or the accumulation of fluids in the pleural cavity or abdominal cavity [2]. Dengue shock syndrome causes a rapid, weak pulse, a narrow pulse pressure of less than or equal to 20 mm Hg or hypotension with cold, clammy skin in the early stage of shock. If not treated promptly, this may soon progress to a more serious form of shock in which pulse and blood pressure become undetectable, resulting in death within 12 to 36 hours after the onset of shock.

There are three phases of symptomatic dengue- Febrile, critical and recovery phase. Majority presents only with febrile phase and then resolution. Few children progress to critical phase, however it is difficult to predict this phase. [2] Depending on the clinical features, patients are classified into three management groups- Group A- can be managed at home, Group B- needs hospital management, Group C- needs emergency management [2]

Current classification of dengue (2021) has patients with symptoms or remains asymptomatic. Further classified as Dengue without warning signs (mild) A, with warning signs and co-morbidity (moderate) B and severe dengue C. A1 is without factors and A2 is with risk factors. B1 is warning signs and symptoms and B2 plus comorbidity and high risk. Severe dengue (C) is C1- shock, C2- bleeding, C3- organ involvement (Acidosis, dyselektrolytemia) [3] It is still unclear why some patients experience severe and life-threatening forms of dengue fever. However, it is believed that several factors associated with the host may increase the likelihood of developing severe dengue fever. These factors include previous exposure to DENV, presence of co-morbidities, and genetic predisposition [3].

The nutritional status of the host has been suggested as a possible predictor of disease progression in dengue patients [4]. There is increasing evidence to suggest that some nutrients have a strong impact on the immune system. One such is nutrient is vitamin D and a deficiency of this vitamin (defined as a serum level below 20 ng/ml) may be a possible risk factor for developing severe dengue illness. However, there have been limited studies evaluating the potential association between vitamin D deficiency and the severity of Dengue disease. [5,6,7,8,9] but the available evidence is scanty and inconsistent.

In this study, we prospectively evaluated the levels of systemic vitamin d (serum 25-(OH)D) in adults who were admitted to our institution with DF, DHF and DSS . The objective of this study was to identify a possible association between low levels of systemic 25-(OH) D and the likelihood of developing severe dengue fever.

Aims and Objectives

Primary Objective: To investigate the potential link between vitamin D deficiencies in relation to the vulnerability of severe Dengue illness this was evaluated at the time of admission in patients diagnosed with DF, DHF or DSS, who were hospitalized.

Secondary Objective: To determine the demographic and clinical factors associated with the level of Serum Vitamin D in Dengue patients.

Materials and Methods

Study Design: This was a single-center, single-group, cross-sectional study conducted at the Department of Medicine, Dr. RMLIMS, Lucknow, between the August 2023 to November 2023. Total 156 patients were recruited taken from wards and ICU. Patients were selected according to inclusion criteria and sampling was done for serum vitamin D., They were included after confirming reports of Dengue positive patients from NS1, IgM and IgG (ELISA). The total study participants were divided into two groups , those with vitamin D <20 ng/ml and those with > 20 ng/ml. Blood sampling was done for other hematological parameters also like Serum Bilirubin, SGOT, SGPT, Alkaline phosphatase, serum albumin, hemoglobin, TLC, platelet count, hematocrit, neutrophil, lymphocyte. Clinical and demographic profile like age, gender and Blood pressure were noted after taking proper consent.

Reports also recorded dengue positivity on the basis of NS1, IgM and IgG. Evaluation was also done in adults hospitalized with Dengue without warning signs, Dengue with warning signs and severe Dengue fever prospectively upon admission.

Inclusion Criteria: The study included patients of either gender who were admitted to the dengue wards, Intensive Care Units (ICUs) and OPDs of Dr. RMLIMS. These patients had tested positive for NS1/IgM dengue and had a platelet count of less than 150,000/ μ L of blood. Participants provided informed written consent to participate in the study.

NS1/IgM dengue detection was carried out by enzyme-linked immunosorbent assay (ELISA) (International Immuno-Diagnostics Inc.). Serum levels of vitamin D were also evaluated by ELISA.

Exclusion Criteria: Those who had previous history of thyroid illness or on antithyroid drugs, any rheumatological condition or taking such treatment, previous musculoskeletal disease, cardiac disease, H/O hepatitis/hematological disease, CKD.

Sample size calculation: A previously published article Iqtadar et al. (2023) [17], mentioned that 37

(38.1%) patients were DF. Now, the sample size calculation formula is given below,

$$n = \frac{(Z_{1-\alpha/2})^2 * p * (1 - p)}{d^2}$$

Given that Z is the standard normal variate at a level of significance (α) i.e. 5% is 1.96, p is the proportion of DF patients (=38.1%), and d is the margin of error i.e. 8%. Putting these values in the above formula

$$n = \frac{1.96^2 * 0.381 * (1 - 0.381)}{0.08^2},$$

$$n = \frac{0.906}{0.0064} = 141.56 \cong 142.$$

After calculation obtain the sample size is n=142. Adding 10% of loss of data then optimum sample size N= n/(1-0.1)= 156.

Statistical Analysis: In the present work, study variables were reported as categorical and continuous variables. For the continuous variable, check the test of normality. All continuous values

were summarized as mean \pm SD. Clinical and demographic characteristics of the study groups were compared using the Student's t-test for continuous variables.

Categorical variables were presented as percentages and compared using the χ^2 statistics or Fisher exact test. A p-value of <0.05 was considered significant. All statistical tests have been considered two-sided. MS Excel and R-4.3.2 statistical software were used for statistical analysis.

Results

Table 1: Gender Distribution (for n= 156) with Mean age= 37 years in > 20 and < 20 ng/ml of vitamin D patients (Resolved) Among total patients, 61% (n=45) and 39 % (n=29) of females were in low vitamin D <20 ng/ml and S. vitamin D >20 ng/ml group respectively.

While males were 49% (n=40) and 51% (n= 42) in vitamin D <20 and >20 group respectively. This graph tells overall low vitamin D <20ng/ml was more prevalent in females more than males.

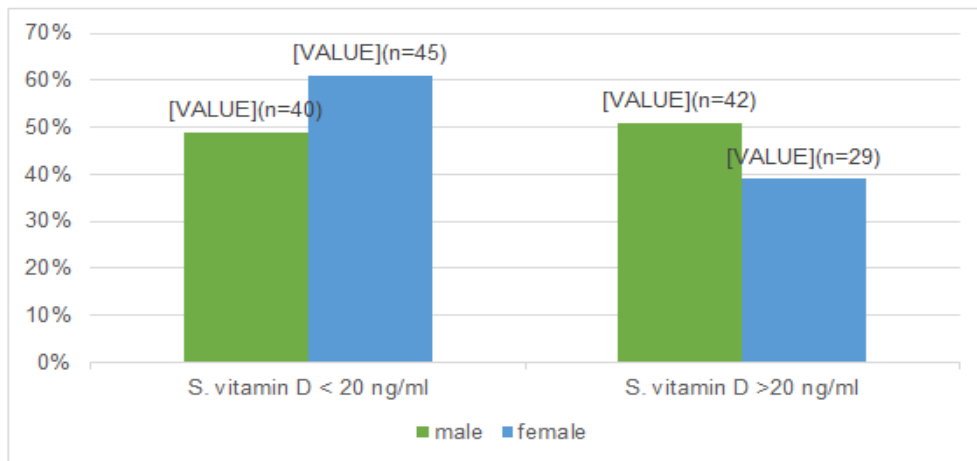


Figure 1:

Table 2: Age Distribution - Patients less than 18 year old (adolescent) and > 18 years (adults)

Age	S. Vitamin D		Total	Chi Square P value
	More than 20 ng/ml	Less than 20 ng/ml		
Adult greater than 18	48	101	149	0.172
Younger less than 18	4	3	7	
Total	52	104	156	

Here the study is showing that patients with age > 18 years had serum vitamin D < 20 ng/ml in n=101 patients and those with age < 18 year had almost same distribution between the two groups.

Table 3: Age Distribution

AGE (in years)	<20 ng/ml (n= 104)	>20 ng/ml(n=52)
11-20	10	8
21-30	32	8
31-40	24	16
41-50	17	12
51-60	13	6
61-70	5	1
71-80	3	0
81-90	0	1

The age group 21 – 30 and 31- 40 years had maximum no of low vitamin D level < 20 ng/ml in dengue patients while those in 31- 40 and 41- 50 years falls in serum vitamin D level > 20 ng/ml.

Table 4: Descriptive Statistics for Hematological and demographic parameters (mean and SD) (N=156)

	N	Minimum	Maximum	Mean	Std. Deviation
Age	156	16	87	36.46	14.428
Serum vitamin D (ng/ml)	156	5	109	18.40	9.680
Bilirubin (mg/dl)	156	.12	4.57	1.2758	.79525
SGOT (U/L)	156	20.0	6469.0	269.615	600.2971
SGPT (U/L)	156	23.00	2584.00	165.5656	287.58129
ALP(U/L)	156	34.0	448.0	125.454	66.6655
S. Albumin (g/dl)	156	1.12	5.85	3.4971	.97207
Hb (gm/dl)	156	7.0	18.3	11.306	2.5031
TLC ($\times 10^3$ per cmm)	156	12.04	21000.00	5414.5772	3448.50431
Plt $\times 10^3/\mu$ l	156	10000	970000	54888.08	81635.180
Hematocrit (%)	156	23.0	56.9	38.422	6.2181
Neutrophil (%)	156	20	83	46.63	11.231
Lymphocyte (%)	156	6	65	35.62	10.635
Pulse (BPM)	156	60	136	95.25	15.137
BP Sys mm Hg	156	74	168	120.65	15.243
BP Dys mm Hg	155	9	100	83.54	12.408

Table 5: Comparison of Hematological and demographic parameters between <20 and > 20 ng/ml serum vitamin D

Variables	S Vitamin D	N	Mean	Std. Deviation	T test p-value
Age	<20	104	36.52	14.556	0.944
	>20	52	36.35	14.310	
Bilirubin mg/dl	<20	104	1.2975	.82705	0.632
	>20	52	1.2325	.73335	
SGOT U/L	<20	104	324.028	724.8140	0.110
	>20	52	160.788	126.7943	
SGPT U/L	<20	104	189.0791	345.80929	0.149
	>20	52	118.5385	80.35521	
ALP U/L	<20	104	131.547	65.3596	0.107
	>20	52	113.269	68.2096	
S Albumin g/dl	<20	104	3.4569	.96253	0.467
	>20	52	3.5775	.99539	
Hb gm/ dl	<20	104	11.235	2.5333	0.614
	>20	52	11.450	2.4596	
TLC per cmm	<20	104	5609.5385	3420.90713	0.320
	>20	52	5024.6546	3503.66962	
Plt per cmm	<20	104	59248.46	97050.715	0.347
	>20	52	46167.31	33400.192	
Hematocrit	<20	104	38.227	6.0381	0.580
	>20	52	38.813	6.6065	
Neutrophil	<20	104	46.98	11.360	0.581
	>20	52	45.92	11.043	
Lymphocyte	<20	104	34.60	10.219	0.089
	>20	52	37.67	11.242	
Pulse BPM	<20	104	94.24	15.372	0.240
	>20	52	97.27	14.590	
BP_Sys mmHg	<20	104	120.50	15.566	0.865
	>20	52	120.94	14.719	
BP_Dys mmHg	<20	103	82.96	13.826	0.419
	>20	52	84.67	8.976	

Above are the results are not statistically significant for the increased severity in Dengue patients like deranged LFT (high bilirubin, elevated SGOT, SGPT), low TLC, low neutrophil count, low serum albumin, LOW

platelet count , low BP<100/90, tachycardia, any bleeding manifestation, ascites, pleural effusion in respect of the level of vitamin D level </> 20 ng/ml.

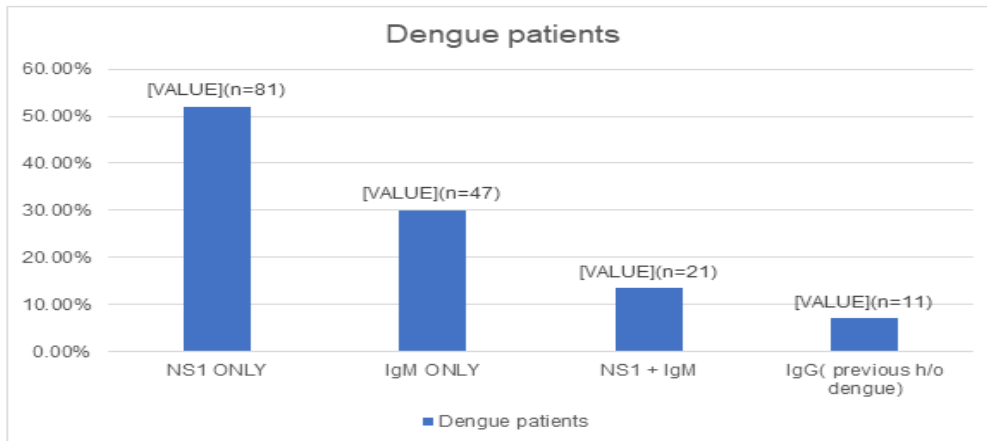


Figure 1: showing the Prevalence of Dengue patients according to new and old ELISA reports

This table is showing dengue patients positive due to NS1 only as 51.90% (n=81), IgM only as 30.10% (n=47), combined NS1 with IgM as 13.46% (n=21). There was h/o previous dengue in 7.05% (n=11).

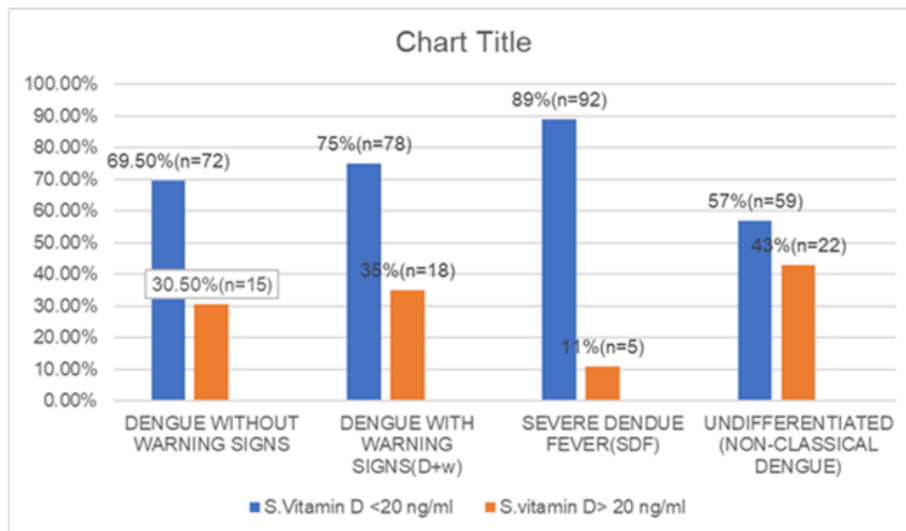


Figure 2 : showing the distribution of < 20 and > 20 ng/ml vitamin D patients among severity of Dengue patients.

In the present study, we evaluated systemic vitamin D, i.e., serum 25-(OH) D levels in adults hospitalized with Dengue without warning signs , Dengue with warning signs and severe Dengue fever prospectively upon admission. A total of 156 patients were taken in the study. Assessment of various clinical characteristics of Dengue positive patients and measurement of vitamin D deficiency was done and following observations were made.

The prevalence of vitamin D deficiency in dengue fever-

Table 6:

	Serum Vitamin D < 20 ng/ml	Serum Vitamin D > 20 ng/ml
Dengue Fever	69.5 %	30.5 %
Dengue hemorrhagic fever	75%	35%
Dengue Shock syndrome	89%	11%

Table 7: To find statistically significant difference in the dengue clinical symptoms between s.vitamin D <20 and >20

Symptoms		Vitamin D Level ≥ 20 ng/ml ≤ 20 ng/ml	Chi Square test (P- value)
Fever	No	8	0.99
	Yes	44	
Rashes	No	46	0.8624
	Yes	6	
Arthralgia	No	29	0.7334
	Yes	23	
Bodyache/headache	No	35	0.4123
	Yes	17	
Diarrhea	No	46	0.2242
	Yes	6	
Epistaxis	No	41	0.8903
	Yes	11	
Malena	No	45	0.9382
	Yes	7	
Hematemesis	No	50	0.6587
	Yes	2	
Pain abdomen/heaviness	No	38	0.2727
	Yes	14	
Vomiting/ nausea	No	35	0.8064
	Yes	17	
Dizziness	No	51	0.4943
	Yes	1	

Table 8 : Comparative Ultrasound findings in < 20 and > 20 vitamin D deficient patient. (n=156).

This figure is showing increased complications due to capillary leakage in low vitamin D < 20 ng/ml group. Ascites and ascites with pericholecystic fluid were reported in 60.8% (n=14) and 67.7%(n=23) of dengue patients with vitamin D < 20 ng/ml.

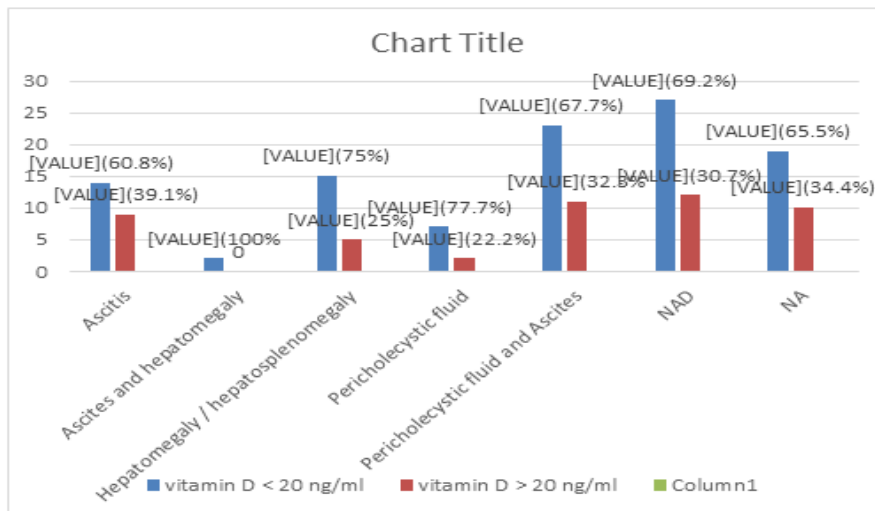


Figure 4:

Table 9 : Radiological parameters by vitamin D deficiency status

1. Chest X-Ray

	S Vitamin D		Total	P- value
	Greater than 20	Less than equal to 20		
NA*	12	17	29	
NAD**	24	43	67	0.2676
pleural effusion	16	44	60	
Total	52	104	156	

* Not available, **not significant

2. Ultrasound Abdomen

	S Vitamin D		Total	Chi Square test (Yates)
	Greater than 20	Less than equal to 20		P value
Ascites	9	14	23	0.6297
Ascites and hepatomegaly	0	2	2	
Hepatomegaly /hepatosplenomegaly	5	15	20	
NAD	12	27	39	
Pericholecystic fluid	2	7	9	
Pericholecystic fluid and Ascites	11	23	34	
NA	10	19	29	
Total	52	104	156	

In low vitamin D patients (< 20 ng/ml) radiological findings were more, indicating increased capillary leakage, like pleural effusion and ascites, because of the difference in sample size likely

(P-value= 0.6297). Where hepatomegaly > pericholecystic fluid + ascites > ascites, more in Vitamin D < 20 ng/ml patients.

Therefore, more derangements were seen in patients with vitamin D <20 ng/ml, stating that severity of symptoms/ lab findings / radiological findings was more in low vitamin D patients.

Those with Vitamin D < 20ng/ml had maximum duration of stay of 23 days and minimum of 5-7 days. Those with DSS had maximum duration of stay in hospital. Patients were also admitted in ICU for MODS and there were some cases of altered sensorium which needed CSF analysis and NCCT Brain. CSF analysis was normal and NCCT brain was WNL. Those with vitamin D < 20 ng/ml had 89% and 11% (vit. D >20 ng/ml) admission in ICU for DSS.

Discussion

It was interpreted that the severity of Dengue was more in low vitamin D patients and it was probably due to reduced immunity in such patients.

Of the total (n=157), 47.4 % (n=74) were female and 52.6 % (n=82) were in age group 18- 87 years, and 15.9% evolved to be had worse outcome.

Bodyache or headache was in (37.2%, n=58), arthralgia in (46.2%, n=72) though very common presentation in either group (with p-value= 0.4123, 0.7334) was present more in vitamin D deficient patient.

Other presentations like bleeding manifestations were taken in account separately like Malena were in 14.7% (n= 23), hematemesis 3.8% (n= 6), and epistaxis (n=34). Pain abdomen or heaviness was reported in 21.8% (n=34). According to this cohort analysis, which aligns with previous studies [10,19,20,21,22],147 (93.6%) patients had significantly increased levels of both AST and ALT liver enzymes when they were admitted. The acute phase of DENV infection is also associated with liver damage, which is believed to be caused by

various factors, including the virus directly inducing apoptosis in hepatocytes, hypoxic damage due to fluid leakage, oxidative stress or immune mediated injury (23,24,25).

Nausea and vomiting were reported in 31.4%, n=49. Some patients also reported previous dengue infections 7.1%, n=11.

In the present study, patients with vitamin D deficient had more likely to have severe presentation like majority had bleeding manifestations.

In this analysis, LFT were markedly deranged, 104 patients has raised SGOT/SGPT in vitamin D < 20 ng/ml and 52 patients in vitamin D >20 ng/ml. Some patients also had septicemia with TLC of 21000/cmm.

USG abdomen showed ascites and pericholecystic fluid in 21.8%, n= 34. Ascitis alone was reported in 14.7%, n=23. Capillary leakage was defined by hematocrit and pleural effusion 38.5%, n= 60 also. Hematocrit was minimally recorded 23 and maximally 56.9%. Platelet was minimum <10000 and maximum 97000.

In this study , the role of vitamin D deficiency in Dengue severity was not statistically significant, There are several studies [6, 7, 8, 9, 11,12] which are showing reports i.e., higher serum 25-(OH)D has more severe cases of DHF/DSS.[5,8]

There are clinical trials investigating the utility of vitamin D in preventing severe dengue, though are limited.[26] The cause-and-effect relation of low vitamin D to the severity of Dengue can be the possibility. Vitamin D influences the expression of DENV entry receptor, dendritic cell specific intercellular adhesion molecule-grabbing non-integrin (DC_SIGN) and FCyRIIA in immune cells [6,13,14]. The results are not statistically significant to ≤ 0.05 in multiple separate analysis.

Limitations of The Study: Our study has limitations like there was no control group and not included non-dengue febrile patients or healthy volunteers.

Conclusion

This study concludes that vitamin D deficiency is not significantly associated in deciding the severity of Dengue fever patients. But the study has shown severity of symptoms more in low vitamin D group. Therefore, one of the important management parts in dengue patients should be supplementation of vitamin D in all such patients regardless of the severity. However accurate level must be measured in each dengue patients. This will help in relief of symptoms like headache, bodyache and arthralgia also.

Therefore creating awareness for the normalisation of vitamin D in general population should be promoted. This will help in reducing the prevalence of severity in tropical diseases like Dengue. Mass programme for Vitamin D investigation should be done by health and hospital authority.

Author Contribution

Conceptualisation: Jyoti Verma, Methodology: Jyoti Verma and Ankit Singh, Statistical analysis: Anurag Pathak, Review and editing: Jyoti verma, Ankit Singh

All authors contributed to the article equally.

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