

Vitamin D Deficiency and its Propensity for Severe Dengue Fever: A Prospective Observational Study in a Tertiary Care Centre, Odisha, India

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ABSTRACT

Introduction: In addition to being necessary for maintaining the body's calcium and phosphorus levels and regulating bone metabolism, vitamin D (vit D) is regarded as a potent immunomodulator that influences both innate and adaptive immune responses and is crucial for pathogen defence systems. There is growing interest in vitamin D's potential role in Dengue Virus (DENV) infection.

Aim: To ascertain whether vitamin D deficiency is related to severe dengue disease in individuals diagnosed with Dengue Fever (DF) or its sequelae.

Materials and Methods: The current study was a single-centre, cross-sectional study, including 172 patients diagnosed with dengue after obtaining written informed consent. The diagnosis was confirmed by a Non Structural Protein 1 (NS1) test or a dengue specific IgM or IgG antibodies test using Enzyme Linked Immunosorbent Assay (ELISA). A structured questionnaire was used to collect information from the patients, including demographic factors, vital signs, laboratory parameters, and radiology findings. Vitamin D levels were measured in all patients using spectrometry and were divided into two groups: deficient

(≤ 20 ng/mL) and sufficient (> 20 ng/mL). Data collected were analysed using Statistical Package for Social Sciences (SPSS) version 27.

Results: The mean age of the study participants was 37 years, and most of the patients were male 102 (59.3%). With respect to dengue infection, 49 (28.5%) had DF, 84 (48.8%) had Dengue Haemorrhagic Fever (DHF), and the remaining 39 (22.7%) had Dengue Shock Syndrome (DSS). According to vitamin D status, 118 (68.6%) had insufficient vitamin D levels, while the remaining 54 (31.6%) had sufficient vitamin D. The proportion of females in the vitamin D deficient group was significantly higher (82.9%) compared to the vitamin D sufficient group (17.1%) (p -value=0.001). The severe forms of dengue infection, such as DHF and DSS, were found to be significantly higher in the vitamin D deficient group compared to the sufficient group, with a p -value of 0.041.

Conclusion: The study found a significant association between vitamin D levels and the severity of DF. The use of vitamin D as a therapeutic measure for all forms of DF is supported by the study findings.

Keywords: Cholecalciferol, Dengue shock syndrome, Flaviviral infection

INTRODUCTION

Dengue is a flaviviral infection transmitted by *Aedes* mosquitoes, predominantly found in tropical and subtropical regions. The Dengue Virus (DENV) comprises four serotypes: DENV1, DENV2, DENV3, and DENV4 [1-3]. An initial infection with any of these serotypes is generally moderate or asymptomatic; however, a subsequent infection with a different serotype may lead to severe dengue symptoms. Around 50 million individuals in dengue-endemic countries contract DENV infection yearly, with Asia representing 70% of the cases. Annually, India endures significant dengue outbreaks that impact the healthcare system. Despite the global mortality rate from dengue being relatively modest at 1%, a lack of prompt intervention can elevate it to 20% [1,3,4].

Dengue infection can present clinically as asymptomatic or exhibit a spectrum of symptoms known as DF. The symptoms of DF vary from a mild flu-like illness to a severe manifestation called DHF. Haemorrhagic manifestations, such as spontaneous bleeding, a significant decrease in platelet count, and heightened vascular permeability, indicated by ascites, pleural effusion, or increased haemoconcentration, are characteristics of DHF [5,6]. If not promptly addressed, DHF can advance to a critical condition known as DSS [7,8]. A diminished pulse, reduced pulse pressure, or hypotension accompanied by chilly, clammy skin during the initial phases of shock indicates DSS, which occurs following a decrease

in blood pressure. If not treated promptly, the condition may deteriorate, resulting in a more severe form of shock characterised by undetectable blood pressure and pulse, potentially leading to significant complications [8].

According to the World Health Organisation, individuals infected with DENV are categorised as either asymptomatic or symptomatic. Dengue is classified into three categories: mild (asymptomatic dengue), moderate (dengue with symptoms), and severe dengue (dengue with complications). Mild dengue is further divided into cases with and without risk factors. Moderate dengue is further classified as having warning signs and comorbidities. Severe dengue encompasses three types of complications: shock, haemorrhage, and organ involvement, including acidosis and electrolyte imbalance [9].

The cause of severe and potentially deadly DF in certain individuals is not well understood. However, some host-related factors are thought to increase the risk of presenting with severe DF. These factors include co-morbidities, genetic predisposition, and prior DENV exposure [8]. Research is ongoing to identify new, potentially modifiable host risk factors that could prevent the progression of severe disease without the need for DENV-specific antiviral medication.

The dietary status of individuals with dengue has been suggested as a potentially significant determinant of disease progression [10,11].

There is increasing evidence indicating that nutrition substantially influences the immune system and may be linked to dengue infection [10,11]. Vitamin D is one of the nutrients whose deficiency may elevate the risk of acquiring a serious dengue infection. It is believed that vitamin D could mitigate the severity of the illness by reducing the number of infected cells, particularly monocytic cells, thereby decreasing proinflammatory cytokines such as interleukins (IL-6 and IL-1 β) and Tumour Necrosis Factor-alpha (TNF- α) [12]. Nonetheless, information regarding the association between serum vitamin D levels and the severity of dengue infection is sparse [13-15].

This study was therefore conducted to determine the association between vitamin D deficiencies and severe dengue illness among patients diagnosed with DF or its complications.

MATERIALS AND METHODS

The current study was a single-centre, prospective observational study conducted at the Department of Medicine of a tertiary care centre in Odisha, India, between July 2024 and November 2024. The study was approved by the institutional ethics committee (letter no. IEC/PRMMC/2024/24/16).

Inclusion and Exclusion criteria: Patients diagnosed with dengue from the inpatient department and Intensive Care Unit (ICU) were recruited into the study after providing written informed consent. The diagnosis was confirmed by an NS1 test or a dengue-specific IgM or IgG antibody test using ELISA. Patients who tested positive for either NS1 or IgM antibodies, along with a platelet count of less than 100,000/ μ L, were included in the study. Furthermore, in accordance with guidelines, each patient was evaluated and categorised as having DF, dengue haemorrhagic fever, or DSS [2]. Those with a previous history of thyroid illness or who were on antithyroid drugs, rheumatological conditions or receiving treatment for the same or similar conditions, musculoskeletal disorders, life-threatening cardiac diseases, histories of hepatitis, haematological diseases, and patients with chronic kidney disease were excluded from the study.

Sample size calculation: A recent study by Dissanayake S et al., reported a prevalence of vitamin D deficiency of 65% among dengue patients. Using this proportion as the effect size, alongside a power of 80%, an alpha value of 0.05, a margin of error of 8%, and a 95% confidence interval, the minimum sample size was calculated to be 137. In this study, a total of 172 patients diagnosed with dengue were recruited [16]. The sample size was calculated using the OpenEpi open-source software [17].

Study Procedure

A structured questionnaire was used to collect information from the patients. General information such as age and gender, presenting symptoms and signs, vital signs including pulse and blood pressure, and laboratory parameters such as complete blood count, liver function tests, and haemoglobin levels were measured and documented for each patient. Radiological investigations, including chest X-rays and abdominal ultrasonography, were performed wherever indicated, and the results were noted. Vitamin D levels were measured using spectrometry for each patient and divided into two groups: deficient (≤ 20 ng/mL) and sufficient (> 20 ng/mL) [18].

STATISTICAL ANALYSIS

The data collected were analysed using SPSS version 27 (IBM Corporation, Armonk, USA). The variables were presented as categorical (represented as frequencies and percentages) and continuous (represented as means and standard deviations). The normality of continuous variables was assessed using the Shapiro-Wilk test. The Chi-squared test or Fischer's exact test was used to assess associations between two categorical variables. The unpaired Student's t-test or Mann-Whitney U test was employed to compare means between two groups depending on the distribution

of continuous variables. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The study included 172 patients diagnosed with dengue in the analysis. The mean age of the study participants was 37 ± 5.8 years, ranging from 18 to 84 years. Eighty-one (47.1%) participants were between 31 and 50 years old, followed by those under 30 years at 59 (34.3%). Most of the study participants were male (102, 59.3%).

In terms of dengue infection, 49 (28.5%) had DF, 84 (48.8%) had DHF and the remaining 39 (22.7%) had DSS. Of the total study participants, 91 (52.9%) tested positive only for NS1, 49 (28.5%) tested positive only for IgM, while 16 (9.3%) were positive for both NS1 and IgM. Additionally, 13 (7.5%) were positive for both IgM and IgG, and the remaining 3 (1.7%) tested positive for all three. Regarding vitamin D status, 118 (68.6%) had deficient levels, while the remaining 54 (31.4%) had sufficient vitamin D. The proportion of females in the vitamin D deficient group was significantly higher (82.9%) compared to the vitamin D sufficient group (17.1%) (p-value=0.001). Age distribution was comparable in both groups [Table/Fig-1].

Variables	Vit D deficient n (%)	Vit D sufficient n (%)	Total n (%)	p-value
Gender				
Male	60 (58.8)	42 (41.2)	102 (59.3)	0.001
Female	58 (82.9)	12 (17.1)	70 (40.7)	
Age group				
≤30 years	41 (69.5)	18 (30.5)	59 (34.3)	0.604
31-50 years	53 (65.4)	28 (34.6)	81 (47.1)	
>50 years	24 (75.0)	8 (25.0)	32 (18.6)	
Symptoms				
Fever	113 (96.1)	52 (96.3)	165 (96.2)	0.348
Body ache	97 (82.2)	45 (83.3)	142 (82.6)	
Headache	67 (56.8)	22 (40.7)	89 (51.7)	
Vomiting	39 (33.1)	15 (27.8)	54 (31.4)	
Abdominal pain	27 (23.3)	12 (22.0)	39 (22.9)	
Arthralgia	19 (15.7)	9 (17.0)	28 (16.1)	
Bleeding	52 (44.1)	11 (20.1)	63 (36.6)	

[Table/Fig-1]: Age and gender distribution of study participants according to Vitamin D status (N=172).

Most patients experienced fever (96.1% vs 96.3%) and body aches (82.2% vs 83.3%), regardless of vitamin D status. Headache was the next most common symptom, followed by vomiting, abdominal pain, and arthralgia, with patients experiencing these symptoms in almost equal proportions in both the vitamin D deficient and sufficient groups. Patients with symptoms such as epistaxis, haematemesis, gum bleeding, melaena, and rectal and vaginal bleeding were grouped as having bleeding manifestations. Bleeding manifestations were significantly higher in the vitamin D deficient group compared to the sufficient group (p-value < 0.001) [Table/Fig-1].

The severe forms of dengue infection, such as DHF and DSS, were found to be significantly higher in the vitamin D deficient group compared to the sufficient group, with a p-value of 0.041 [Table/Fig-2]. The description of laboratory parameters in both groups is shown in [Table/Fig-3]. None of the parameters were significantly different between the two groups except for the alkaline phosphatase

Infection	Vit D deficient n (%)	Vit D sufficient n (%)	Total n (%)	p-value
DF	28 (23.7)	21 (38.9)	49 (28.5)	0.041
DHF/DSS	90 (76.3)	33 (61.1)	123 (71.5)	

[Table/Fig-2]: Association of dengue infection with Vitamin D status (N=172).

Parameters	Vit D deficient (mean±SD)	Vit D sufficient (mean±SD)	p-value
Bilirubin (mg/dL)	1.41±0.86	1.24±0.42	0.163
SGOT (U/L)	298.54±468.26	183.32±128.61	0.077
SGPT (U/L)	206.46±331.92	122.62±86.83	0.069
ALP (U/L)	136.32±66.42	112.32±68.56	0.031
S. Albumin (g/dL)	3.28±0.95	3.56±0.98	0.081
Hb (gm/dL)	11.24±2.54	11.52±2.33	0.499
TLC per cu mm	6273.72±3641.79	6105.74±3522.59	0.777
Platelet per cu mm	54698.46±86321.55	58982.33±35211.54	0.725
Haematocrit (%)	40.14±6.56	39.46±6.74	0.535
Neutrophil count per cu mm	52.98±10.49	53.72±10.09	0.664
Lymphocyte count per cu mm	39.10±11.53	38.19±11.12	0.627
Pulse rate (bpm)	96.74±14.93	95.27±14.57	0.546
SBP in mm of Hg	126.52±15.15	122.67±14.32	0.117
DBP in mm of Hg	78.96±11.23	81.02±9.45	0.243

[Table/Fig-3]: Comparison of different laboratory parameters with Vit D status (N=172).

level, which was significantly higher in the vitamin D deficient group compared to the sufficient group.

The complications arising from dengue infection in the study participants are depicted in [Table/Fig-4]. As there were some missing data for a few patients, the total number varied for each complication in the analysis. Pleural effusion was observed in a significantly higher proportion of the vitamin D deficient group (p-value=0.010). Other complications such as ascites, hepatic/splenomegaly, and pericholecystic fluid accumulations were comparable in both groups. Any complications were more frequently observed in the vitamin D deficient group (72.3%) compared to the sufficient group (37.7%), which was statistically significant (p-value=0.041).

Complications	Vit D deficient n (%)	Vit D sufficient n (%)	Total n (%)	p-value
Pleural effusion (n=136)				
Present	39 (83.0)	8 (17.0)	47 (34.5)	0.010
Absent	56 (62.9)	33 (37.1)	89 (65.5)	
Ascites (n=140)				
Present	19 (73.1)	7 (26.9)	26 (18.5)	0.470
Absent	75 (65.8)	39 (34.2)	114 (81.5)	
Hepato/Splenomegaly (n=140)				
Present	19 (63.3)	11 (36.7)	30 (21.4)	0.610
Absent	75 (68.2)	35 (31.8)	110 (78.6)	
Pericholecystic fluid (n=140)				
Present	30 (78.9)	8 (21.1)	38 (27.1)	0.071
Absent	64 (62.7)	38 (37.3)	102 (72.9)	
Any complications (n=140)				
Present	68 (72.3)	26 (37.7)	94 (67.1)	0.042
Absent	26 (56.5)	20 (43.5)	46 (32.9)	

[Table/Fig-4]: Association between complications of dengue and Vit D status.

DISCUSSION

Vitamin D deficiency was identified as a predictor of dengue severity (DHF/DSS) in this study, as the deficient group exhibited a greater proportion of severe dengue infections compared to the vitamin D sufficient group (76.3% vs 61.1%). A study by Dissanyake S et al., in their case-control study observed that patients with severe dengue infections had very low vitamin D levels (65%), which is similar to the findings of this study [16]. Similarly, Samal S et al., conducted a study in India that found comparable results [19].

A few studies [15,20-22] have proposed the potential role of vitamin D in dengue infection, highlighting its insufficiency as a major risk factor

for severe dengue disease [16,23-24] and suggesting its use as a potential adjuvant in the treatment of dengue patients [12,21,22,25]. Certain research has demonstrated conflicting outcomes, indicating that elevated serum vitamin D levels are correlated with more severe dengue conditions (DHF/DSS) [26].

The potential correlation between vitamin D deficiency and increased vulnerability to severe DENV infection may be elucidated through various mechanisms. Vitamin D, an immunomodulator influencing both the innate and adaptive immune systems, facilitates macrophage differentiation, thereby inhibiting virus replication [14,22,27]. Cytokine production was considerably reduced in dengue-infected macrophages treated with vitamin D compared to those without treatment [28]. Vitamin D also impacts the expression of the DENV entry receptor, Fc Receptor IIA (FCRIIA), in immune cells, as well as dendritic cell-specific intercellular adhesion molecule-grabbing non-integrin [22].

The immunological mechanisms linking 25-(OH)D to the progression and severity of dengue illness remain poorly understood. In an in vitro study utilising different human cell lines exposed to varying doses of 1,25-(OH)₂D₃ followed by infection with DENV serotype 4 (DENV-4), a significant reduction in the percentage of infected cells and a decrease in the production of IL-12p70, IL-6, IL-1β, and TNF-α were observed, with a dose-response relationship evident for 1,25-(OH)₂D₃ [25]. The fundamental immunological processes remain unclear. Alzate A et al., subjected macrophages derived from healthy humans to different doses of 1,25-(OH)₂D₃ and subsequently infected them with DENV-2 [13]. The macrophages that differentiated under elevated concentrations of 1,25-(OH)₂D₃ exhibited diminished DENV infectivity, likely attributable to reduced levels of pro-inflammatory cytokines and decreased expression of receptors necessary for DENV entry into macrophages [28].

Limitation(s)

The main limitation of this study was that it was conducted at a single centre, which may not be applicable to a broader population with diverse genetic backgrounds and dietary variations. Subsequent multicentric research with substantial sample sizes and rigorous analytical or experimental methodologies will yield improved clinical evidence derived from the findings of this study.

CONCLUSION(S)

The study establishes a significant association between vitamin D deficiency and severe dengue. Consequently, an essential aspect of managing dengue patients may be the administration of vitamin D supplements for all individuals, irrespective of disease severity.

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