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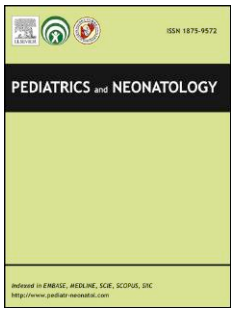

Association between Vitamin D level and Dengue Severity in Children: A Prospective Study in an Eastern Indian City

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Title page

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Abstract:

Background: Dengue fever, a significant health concern in tropical regions, including India, presents in healthcare settings with a disease spectrum ranging from mild to severe illness. Anti-body dependent enhancement (ADE) and non-ADE mechanism such as nutrition (vitamin D, etc.) have been suggested for dengue severity. The relationship between vitamin D, a known immune modulator, and the severity of dengue fever in pediatric patients was investigated.

Method: This prospective study, enrolled 99 children aged 1–18 years with confirmed dengue fever between April 2023 and September 2024. Patients were classified into dengue fever (DF) or those with complications, including, "Dengue with Warning Signs (DWS) and Severe Dengue (SD)." Serum vitamin D levels were measured at admission, and clinical outcomes and laboratory parameters were analyzed. The ROC curve analysis was used to evaluate vitamin D's ability to predict complications.

Results: Patients having DF with complications, i.e., DWS and SD, had lower vitamin D levels (Median 13.5 ng/ml vs. 21.5 ng/ml) than DF patients. Those with vitamin D levels <20 ng/ml had a 3.3-fold higher risk of complications ($p = 0.01$). A vitamin D cutoff of 17.9 ng/ml predicted severity with moderate accuracy (AUC = 0.683). Severe cases required longer hospital stays and more intensive care but had full recovery.

Conclusion: Vitamin D deficiency is linked to greater dengue severity in children. Addressing this modifiable risk factor could help reduce severe outcomes, emphasizing the potential role of supplementation in dengue management.

Key Words: Dengue Severity; Vitamin D Deficiency; Child; Non-ADE Dengue.

Introduction

In 2023, dengue cases increased steeply worldwide, reaching over five million infections and claiming more than 5,000 lives.[1] India has also experienced a significant rise in dengue cases, with reported numbers jumping from 157,315 cases and 166 deaths in 2019 to 289,235 cases and 485 deaths in 2023. The surge was particularly notable in Kerala and the north-eastern states.[2] The surge can be attributed to factors like rapid urbanization, changing climate patterns, and limited vector control measures.[1] Considering the higher fatality of up to 20% among untreated cases,[3] dengue control has immense implications for low-middle income countries with limited access to healthcare.

Dengue fever, caused by the dengue virus (DENV), comes in four distinct serotypes: DENV1, DENV2, DENV3, and DENV4. While a first-time infection with any of these serotypes is usually mild or even symptom-free, subsequent infections with a different serotype can result in more severe forms of the disease.[4] Dengue can present in a wide spectrum of ways, from showing no symptoms at all to developing into serious conditions like dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS). DHF is characterized by haemorrhagic manifestations, thrombocytopenia, and increased vascular permeability, potentially causing hypovolemic shock without prompt treatment. DSS, the most severe form dengue, can rapidly progress to a life-threatening condition that demands immediate medical attention.[4,5]

Despite the availability of a dengue vaccine, its use is limited to specific populations,[6] and though it is approved by Government of India, yet not commercially available.[7] The current management of dengue in India primarily involves supportive care, including the use of antipyretics, fluid replacement, and blood products in severe cases.[8] However, the factors which drive the progression of dengue from a mild to severe and potentially fatal disease have yet to be explored fully. Host factors including prior exposure to DENV, co-morbidities, nutritional status and genetic predisposition are likely to play a role in determining disease severity.[9]

Nutritional status, particularly regarding vitamin D levels, has emerged as a potential modifiable risk factor that could influence the severity of dengue.[10] In one multi-centric study, the prevalence of vitamin D deficiency in the general pediatric population (7–18 years) in India was 26%.[11] Vitamin D, which is known for its immunomodulatory properties,[12] has been suggested as a factor that might impact the progression of dengue illness. The relationship between vitamin D deficiency (VDD) and severe dengue is scarce and inconsistent, however.[13–15]

Understanding the link between vitamin D serostatus and the severity of dengue could help develop both preventive measures and supportive treatment strategies. In this context, this study evaluates the association between serum vitamin D level at the time of admission and likelihood of developing severe forms of dengue.

Methods:

Study Design and Setting: This prospective study was conducted at Pradyumna Bal Memorial Hospital, Kalinga Institute of Medical Sciences, a large tertiary care centre in Bhubaneswar, Odisha, India, over a period of one and half years from April 1, 2023 to September 30, 2024.

Sample Size: Assuming about 130 primary dengue cases (finite population) during the study period, prevalence of 50%, a finite population of 160 (80 x 2-year study period), absolute confidence limits of 5%, and design effect of 1, the sample size was estimated to be 98.

Participants: The study enrolled 99 patients aged 1–18 years, with acute febrile illness and compatible signs and symptoms, suggestive of dengue fever based on the WHO criteria,[3] and cases were confirmed by dengue NS1 antigen and or serum Ig M antibody. All cases were Ig G antibody negative, indicating they were primary dengue cases. Dengue-positive patients who were admitted to pediatric wards, high-dependency units (HDUs) and Pediatric Intensive Care Units (PICUs) were included in the study. Individuals with known chronic illnesses that affects Vitamin D metabolism, such as chronic kidney disease and liver disorders, receiving drugs interfering with vitamin D and calcium metabolism or those receiving Vitamin D supplementation and poor nutritional status were excluded from this study.

Data Collection: After admission, patient demographic information, medical history, physical examination findings, and laboratory test results were documented. Serum 25(OH) D levels were assessed within 24 hours of hospitalization using the electrochemiluminescence assay (ECLIA) method with the COBASe 411 immunoassay system (ROCHE Diagnostics, Germany). The assay has a detection limit in the range of 4.5–210 ng/mL. Our laboratory follows internal quality control twice daily, and it participates in the Randox International Quality Assessment Scheme (RIQAS), an external quality assurance program. Preventive maintenance and equipment calibration were carried out according to the manufacturer's recommendations. New reagent lots were validated prior to use to maintain assay integrity and consistency. Routine laboratory tests, including a complete blood count (CBC), liver function

tests (LFT), kidney function tests (KFT), and coagulation profile, were conducted as part of standard care. Additional imaging, such as radiological imaging of chest and abdomen were performed according to the treating physician's discretion and the patient's condition. The progression of the disease was closely observed, and all outcomes were recorded.

Classification of Severity: The final diagnosis of patients was made based on the severity of the disease, i.e., Dengue Fever (DF), Dengue Fever with Warning Signs (DWS), and Severe Dengue (SD), according to the 2009 WHO guideline.[3] Dengue Fever manifests with common symptoms including fever, headache, malaise and rash. Patients with DWS may experience abdominal pain or tenderness, hepatomegaly, persistent vomiting, or mucosal bleeding due to rapid fall in platelet count, with raised haematocrit signalling a potential for more severe illness. Patients with SD may develop serious complications like severe bleeding, shock, fluid accumulation with respiratory distress, and severe organ damage. For the purpose of this study, serum 25(OH)D levels were categorized as insufficient (<20 ng/mL) and sufficient (≥20 ng/mL) in accordance with the Indian Academy of Pediatrics Revised (2021) Guidelines on vitamin D status classification.[16]

Statistical Analysis: Association between vitamin D levels and dengue severity was assessed by comparing means/medians and chi-square tests. Children were grouped into two categories for analysis: Dengue Fever (DF) and Dengue Fever with Complications (DFC), which included both DWS and SD cases due to sample size limitation.

We used binary logistic regression to identify independent predictors of progression to Dengue fever with complications (DFWC). To minimize bias, variables that directly defined severe dengue were excluded from the model. Remaining candidates were considered if they were biologically plausible and showed at least a modest association in bivariate analysis ($p \leq 0.20$). Age and sex were retained as adjustment variables regardless of significance. The final model included vitamin D, neutrophil-to-lymphocyte ratio (NLR), with age and sex. A receiver operating characteristic (ROC) analysis was conducted to determine the vitamin D level that could predict complications in dengue cases. A p-value of less than 0.05 was considered statistically significant. The data were analysed using SPSS (version 28.0).

Ethical Considerations: Written informed consent and/or assent was obtained from participants and parents according to ethical guidelines. The study was approved by the Institutional Ethics Committee of KIMS via Reference number-KIMS/SLRC/2023/103.

Results:

In the study population, 16 (16.16%) patients were below 5 years without any SD. More cases of DF, DWS, and SD were seen in children above 5 years, but these were not statistically significant ($p = 0.293$). Male to female participant ratio was 1.4:1. Mucocutaneous bleeding was absent in most cases, with no significant differences ($p = 0.323$). Presence of vomiting ($p = 0.001$), abdominal pain ($p < 0.01$) and pleural effusion/ascites ($p = 0.009$) were seen more frequently among the severe forms of the disease (DWS & SD) with statistical significance. None of the DF patients required pediatric intensive care unit (PICU) admission, but 63.6% of SD patients and 10.6% of DWS cases required PICU care ($p < 0.001$), indicating increased PICU needs with increased in disease severity. Only one patient with DWS later on progressed to SD and was managed according to the protocol. Other clinical features showed little statistical significance (Table 1).

The mean durations of hospitalization for DF, DWS and SD patients were 5.32, 5.45 and 10.00 days, respectively, indicating higher duration of hospital stay for SD patients.

Table 2 compares laboratory parameters between DF and DFWC. No significant differences were noted in haemoglobin (Hb), haematocrit, Neutrophil/Lymphocyte ratio(NLR), total leukocyte counts, total platelet counts, serum albumin, C-reactive protein, or blood urea across dengue fever types. However, SGPT ($p = 0.047$) and serum creatinine ($p = 0.017$) showed significant increases in patients with DFWC. The mean vitamin D level in patients with DF was 19.65ng/ml, whereas in patients with DFWC it was 15.18ng/ml. This difference was statistically significant ($p = 0.002$).

The median vitamin D levels among DF and complicated dengue fever patients (DFWC) were 21.5ng/ml and 13.5ng/ml, respectively ($p = 0.002$) as shown in the Supplementary Figure (S1). However, the distribution of the laboratory parameters among dengue patients with sufficient vitamin D level does not significantly differ from that of patients with insufficient levels of vitamin D (Table S1).

When vitamin D sufficiency status was used to predict complications among dengue patients, it was found that patients without sufficient levels of vitamin D (<20 ng/ml) had 3.3 times higher odds (unadjusted) of developing DFWC compared to those with sufficient levels of vitamin D (≥20 ng/ml). The Table also suggests that insufficient Vitamin D level can correctly identify 75.86% dengue fever with complications, but with a lower specificity. With a PPV of 68.75% and a NPV of 60.00%, the vitamin D sufficiency status can moderately predict complications, while the LR+ of 1.56 and LR- of 0.47 suggest modest ability to rule in or to rule out complications based on vitamin D levels (Table 3).

The ROC curve suggests moderate discriminatory ability of vitamin D level in identifying dengue fever with complications (AUC = 0.683 (95%CI: 0.573–0.794). However, the curve's position above the diagonal line (random chance) confirms that vitamin D levels have diagnostic relevance in assessing dengue complication (Figure 1). Duration of hospital stay was significantly longer in severe dengue cases ($p < 0.001$). All of our patients recovered completely. Logistic regression showed that only lower vitamin D levels were independently linked to dengue fever with complications, signifying higher vitamin D levels to be protective in nature (OR = 0.90, $p = 0.004$). The NLR, age and sex were not significant predictors (Table-4).

Discussion

This study explored the connection between serum vitamin D levels and the severity of dengue fever in children. Our findings indicate that lower vitamin D levels are strongly associated with severe forms of dengue, such as DWS and SD. Our study based on primary dengue infection observed that the severe form of dengue might be related to the dengue serotypes as suggested by Bos et al[17] and Aggarwal et al.[18] These findings put in question whether SD is predominantly observed in secondary dengue infections or not and it is suggestive of a non-antibody dependent enhancement (non-ADE) mechanism for dengue severity in primary dengue infections. The study result indicates a potential role of vitamin D as a modifiable risk factor in the clinical progression of dengue in regions like India where dengue is endemic.

Considering the high burden of lower vitamin D serostatus in India,[19] our study has implications for clinical management of dengue cases with vitamin D.

Vitamin D is essential for regulating calcium levels and bone mineral metabolism,[10.19] but its importance in immune regulation has gained considerable attention in recent years. Vitamin D exerts effects on the immune system through the vitamin D receptor (VDR), which is expressed on immune cells, like T cells, B cells, and macrophages.[12] On activation, VDR can modulate the expression of numerous genes regulating the immune response, enhancing the pathogen-fighting capacity of the innate immune system while modulating the adaptive immune response to prevent excessive inflammation.

In the context of viral infections, vitamin D influences the expression of antimicrobial peptides such as cathelicidins and defensins, which can directly inhibit viral replication.[20] Additionally, vitamin D can reduce the risk of cytokine storm by balancing the pro-inflammatory and anti-inflammatory cytokines, a phenomenon implicated in severe viral illnesses, including dengue. The immunomodulatory effects of vitamin D are particularly relevant in the pathogenesis of dengue, where both viral load and the host's immune response contribute to disease severity.[12]

Vitamin D influences dengue outcomes through multiple pathways. It modulates cytokine responses, enhances antiviral peptides, and maintains endothelial integrity to reduce vascular leakage. It also regulates inflammation via vitamin D-induced microRNAs. Clinically, low vitamin D levels correlate with greater disease severity, supporting biological plausibility despite limitations in establishing causality.[21]

In this study, no severe dengue cases were found among children under five, like Mishra et al's study in the same region.[22] Our observation that patients with severe dengue had significantly lower serum vitamin D levels compared to those with milder forms of the disease, is consistent with research from Sri Lanka.[13] The Sri Lankan study found that the likelihood of having vitamin D deficiency (VDD) among children with DHF/DSS was higher (OR: 3.65; 95% CI: 1.461, 9.102, $p < 0.006$) than that in their healthy counterparts. Another study from Colombia involving patients between 5 and 86 years revealed that compared to vitamin D levels of ≥30 ng/mL the adjusted odds of progression to DHF/SD were 0.44 for 20–30 ng/ml and 0.13 for <20 ng/ml, respectively, with a significant trend ($p = 0.003$).[15] In another study from Patna, India, the authors have found that the mean serum

vitamin D level was 20.36 ng/ml in children with DF , where as in children with DWS and SD had a mean serum vitamin D level of 16.94 ng/ml and 11 ng/ml, respectively, which was statistically significant according to the dengue fever grading indicating that the lower the serum vitamin D level, the higher was the severity of dengue fever.[23] A study from Eastern India showed that vitamin D deficiency was strongly linked to severe dengue in children, while sufficiency offered protection.[24] Similarly In adults, Sadarangani et al from Singapore found that low 25(OH)D was associated with severe bleeding, independent of thrombocytopenia, suggesting a role beyond platelet counts.[25] Such variability may reflect individual differences in vitamin D responsiveness, as described by Carlberg and Haq's concept of the "personal vitamin D response index," which highlights genetic and epigenetic influences on vitamin D signaling.[26]

In contrast to our findings for primary dengue cases among children, a study comprising pediatric and adult populations from Pune, India observed significantly higher vitamin D concentrations in dengue fever and dengue haemorrhagic fever cases as compared to healthy individuals ($p < 0.005$ and $p < 0.001$), and patients with secondary DHF had significantly higher vitamin D levels compared to those with secondary DF ($p < 0.05$).[27] It is possible that elevated levels of vitamin D with defective vitamin D receptor signaling may alter the VDR-mediated immune function in secondary DF illness.[28]

No significant differences were observed in clinical or laboratory parameters between patients with sufficient and insufficient vitamin D levels. However, lower vitamin D was significantly associated with severe dengue. This likely reflects the multifactorial nature of dengue pathogenesis, where individual lab values may not fully capture the complex interplay of factors driving disease severity. Using a broader clinical grouping (DF and DFC), our analysis suggests that low vitamin D may contribute to overall disease severity, even if it is not directly linked to specific clinical or laboratory markers.

Our findings are also consistent with prior research that has linked vitamin D deficiency with worse outcomes in various viral infections, including hepatitis C, influenza, Covid-19, and HIV.[29] In dengue, the progression from dengue fever to severe dengue is often marked by a

dysregulated immune response, characterized by excessive inflammation and vascular permeability, leading to complications such as plasma leakage, shock, and organ failure.[12] The lower vitamin D levels in patients with severe dengue may contribute to dysregulated immune response, exacerbating the severity of the disease. Given the role of vitamin D in maintaining endothelial integrity[30] and modulating the immune response,[12] its deficiency may increase vascular permeability, elevating the risk of severe complications like DHF and DSS. Additionally, its influence on pro-inflammatory cytokine production may explain the link between low vitamin D levels and severe dengue outcomes driven by cytokine storms.

However, the studies on this topic are limited and somewhat inconsistent. It is suggested that VDR gene variations could influence susceptibility to severe dengue, indicating a complex relationship between vitamin D and dengue.[31]

Our study identified 17.90 ng/ml as a key vitamin D cutoff for predicting dengue severity, with 68.29% sensitivity, 67.24% specificity, and an 84.2% positive predictive value, linking higher levels to milder and lower levels to severe disease. This may guide targeted intervention especially in resource-poor settings using vitamin D levels as a biomarker.

Strengths of the Study: This study from Eastern India prospectively examines the relationship between vitamin D levels and the severity of dengue in children. Using standardized WHO criteria, this study ensured clinical relevance and comparability with other research. Identifying a practical vitamin D cutoff for predicting severity using ROC curve offers a valuable tool for early risk stratification, which is crucial to improving outcomes in vulnerable pediatric patients. With widespread vitamin D deficiency in India, this study underscores the significance of addressing nutrition as part of dengue management.

Limitations of the Study: The limited sample size and single-center design of the study setting may limit the generalizability of the findings, requiring larger, multi-center studies for validation. As an observational study, causality cannot be established, so the association between vitamin D serostatus and dengue severity may reflect either a contributing factor or a consequence of severe illness. Furthermore, factors like sunlight exposure and dietary intake, which influence vitamin D levels, were not accounted for, potentially confounding the results.

Conclusion: This study provides evidence that lower serum vitamin D levels is associated with increased severity of dengue fever in pediatric patients, specifically among schoolchildren. An optimal vitamin D cut-off level of 17.90 ng/ml may differentiate dengue fever from dengue fever with complications, which adds to the growing body of literature suggesting that non-

ADE mechanisms such as vitamin D deficiency may contribute to the pathogenesis of severe dengue. However, further research is needed to validate these findings and assess the potential benefits of vitamin D supplementation as a preventive strategy in dengue-endemic areas. Given the high burden of dengue in India and other tropical countries, addressing vitamin D deficiency could be a simple, cost-effective intervention to reduce the risk of severe outcomes and improve management of this challenging disease.

Conflicts of Interest Statement: None to declare.

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Tables

Table 1: Demographic and Clinical Characteristics of Dengue patients

Variable	Dengue Fever (DF) (N=41)	DWS (N=47)	SD (N=11)	DFWC (DWS+SD) (N=58)	*p*-value*
Age > 5 Years	82.9%	80.90%	100%	84.52%	0.836
Male Sex	60.98%	57.45%	54.55%	56.90%	0.685
Presence of Mucocutaneous Bleeding	0%	4.26%	0%	3.45%	0.510
Presence of Vomiting	36.59%	76.60%	54.55%	72.42%	<0.001
Presence of Hepatomegaly	14.63%	19.15%	27.27%	20.69%	0.442
Presence of Abdominal Pain	7.32%	57.45%	72.73%	60.35%	<0.001
Presence of Pleural Effusion/ Ascites	0%	10.64%	27.27%	13.79%	0.019
I.V. Fluid Administered	80.49%	89.36%	100%	91.38%	0.114
Paediatric Intensive Care Unit Admission	0%	10.60%	63.60%	20.65%	0.001
Shock	0%	0%	45.46%	8.62%	0.075
Blood Product Transfusion Required	0%	0%	0%	0%	0.999
Duration of Hospital Stay > 5 Days	43.9%	48.90%	81.80%	55.14%	0.079
Recovery of patient	100%	100%	100%	100%	0.999

DWS: Dengue Fever with Warning Signs, SD: Severe Dengue, I.V.=Intra-Venous
DFWC: Dengue Fever with Complications

*Chi-square test results for comparison between DF and DFWC

Table 2: Distribution of Laboratory parameters (Mean±SD) between various dengue fever types

Variable	DF (N=41)	DWS (N=47)	SD (N=11)	DFWC(N=58)	p-value*
Hb (gm/dL)	11.73 ± 1.41	12.18 ± 1.82	11.5 ± 2.39	12.05 ± 1.94	0.347
Neutrophil (%)	61.27 ± 15.44	52.77 ± 16.71	52.45 ± 18.48	52.71 ± 16.89	0.012
Lymphocyte (%)	31.05 ± 14.5	39.0 ± 16.19	37.91 ± 17.08	38.79 ± 16.21	0.016
N/L Ratio	3.25 ± 3.58	1.98 ± 1.99	2.35 ± 2.63	2.05 ± 2.1	0.059
Total Leukocyte Count (per cubic mm)	4985 ± 2844	4709 ± 3176	4601 ± 2418	4689 ± 3028	0.624
Total Platelet Count (per cubic mm)	163522 ± 71624	134647 ± 75874	132273 ± 89356	134197 ± 77766	0.059
Haematocrit (%)	36.51 ± 4.49	37.11 ± 5.38	36.15 ± 7.34	36.92 ± 5.74	0.698
Creatinine (mg/dL)	0.51 ± 0.15	0.59 ± 0.17	0.7 ± 0.37	0.61 ± 0.22	0.017
SGOT(IU/Litre)	79.98 ± 51.76	150.83 ± 115.08	1,180 ± 2,325	346 ± 1060	0.062
SGPT (IU/Litre)	45.5 ± 28.82	82.07 ± 81.68	458.52 ± 877.97	153 ± 403	0.047
Serum Albumin (gm/dL)	3.83 ± 0.43	3.75 ± 0.45	3.56 ± 0.6	3.72 ± 0.48	0.232
CRP (mg/L)	17.91 ± 29.13	17.87 ± 25.19	16.69 ± 21.6	17.64 ± 24.38	0.961
Urea (mg/dL)	21.75 ± 8.64	22.26 ± 7.51	29.54 ± 23.27	23.64 ± 12.2	0.398
Vitamin D (ng/ml) (On Admission)	19.65 ± 8.13	15.38 ± 6.19	14.33 ± 4.31	15.18 ± 5.85	0.002

(DF: Dengue fever, DWS: Dengue with warning signs, SD: Severe dengue, CRP: C-Reactive protein, SGOT: Serum Glutamic Oxaloacetic Transaminase, SGPT: Serum Glutamic Pyruvic Transaminase, N/L: Neutrophil/Lymphocytic ratio)

*p-value comparing mean between DF and DFWC using independent t test

Table 3: Prediction of Complications in Dengue fever by Vitamin D sufficiency level

	DFWC	Dengue Fever	Unadjusted OR (95%CI)	p-value
Patient with Vit. D < 20 ng/ml (n=64)	44 (75.86%)	20 (48.80%)	3.30 (1.40-7.79)	0.01
Patient with Vit. D ≥ 20 ng/ml (n=35)	14 (24.14%)	21 (51.20%)		

Sensitivity (Se): 75.86%, Specificity (Sp): 51.22%,
Positive Predictive Value (PPV): 68.75%, Negative Predictive Value (NPV): 60.00%,
Positive Likelihood Ratio (LR+): 1.56, Negative Likelihood Ratio (LR-): 0.47

Table 4: Binary Logistic Regression Examining Factors Associated with Dengue Fever with Complications (N = 99)

Predictor	**aOR**	**95% CI for OR**	***p*-value**
Neutrophil-to-Lymphocyte Ratio	0.87	0.74 – 1.03	.105
Age group (<5vs. ≥5)	0.84	0.26 – 2.71	.768
Sex (Female vs. Male)	1.67	0.67 – 4.18	.272
Vitamin D (ng/ml)	0.90	0.84 – 0.97	.004**

Note. aOR = adjusted odds ratio. CI = confidence interval. ** p < .01

Figure 1: ROC Analysis of Serum Vitamin D Level for Predicting Severe Dengue