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Review Article

Association between Vitamin D and Dengue Severity: A Systematic Review and Meta-Analysis Protocol

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A B S T R A C T

Purpose: To understand how vitamin D influences the severity of dengue fever as per WHO 1997 and WHO 2009 classification, as many studies have shown ambiguity in this respect

Method: The review has been registered in PROSPERO with registration number CRD420251003676 and will be carried out in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2020 standards. Peer-reviewed literature will be used to identify the studies. The literature search will be conducted using the following databases: Web of Science, PubMed, PubMed Central, and Scopus. Additionally, the academic search engine - Google Scholar will be searched.; two reviewers independently will screen the titles and abstracts of studies based on predefined eligibility criteria. The other two reviewers will assess the quality based on the Joanna Briggs Institute quality parameters. Heterogeneity among the included studies will be examined using statistical measures such as I^2 and Cochrane Q statistics. Sensitivity and subgroup analysis will be used to assess the findings' reliability. If there are more than ten articles, a funnel plot test will be employed to assess publication bias.

Conclusion: This systematic review and meta-analysis, in the absence of multi-centric studies, will explore whether Vitamin D deficiency worsens dengue outcomes and if supplementation could help. The findings from this study could help shape future treatments and improve patient care. In areas where dengue is endemic, our systematic review and meta-analysis will assist in pinpointing research gaps and direct future studies and public health initiatives.

Keywords: Dengue Fever, Dengue Haemorrhagic Fever, Vitamin D

Introduction

Dengue virus 1, 2, 3, or 4 (DENV1–4) are the four related single-stranded RNA viruses of the genus Flavivirus that cause dengue, an acute fever illness. Protection against three out of four dengue virus types is temporary.[1] Dengue is usually a self-limiting illness, meaning most people recover without complications. This milder form is known as dengue fever (DF). However, in some cases, the infection can become more severe, leading to dengue haemorrhagic fever (DHF). DHF is marked by increased leakage of plasma from blood vessels, which can cause fluid to accumulate in areas like the abdomen, lungs, and other tissues - often referred to as "third spaces". This leakage is temporary and is believed to result from an abnormal immune response that triggers excessive cytokine production, commonly known as a cytokine storm. Instead of causing inflammation or direct damage to blood vessels, this response makes the tiny blood vessels more permeable, disrupting normal blood clotting mechanisms and increasing the risk of bleeding.[2]

Based on specific criteria, the WHO 2009 divided dengue severity into three categories: severe dengue, dengue with warning signs, and dengue without warning signs.[3] In contrast, the WHO 1997 divided dengue severity into four categories: undifferentiated dengue, classical dengue, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS).[4]

As of April 30, 2024, the World Health Organization (WHO) has recorded over 7.6 million dengue cases worldwide. Among these, 3.4 million have been confirmed, with more than 16,000 classified as severe, and over 3,000 deaths reported.[5] Currently, dengue is actively spreading in 90 countries, though not all are fully accounted for in official reports. Additionally, many countries where dengue is common lack robust detection and reporting systems, meaning the actual global impact of the disease is likely much higher than the reported figures suggest.[6]

The rise in dengue outbreaks and its spread to new regions can be attributed to several key factors. In endemic areas, dengue transmission seasons are starting earlier and lasting longer, increasing the risk of large-scale outbreaks. A previous dengue virus (DENV) infection increases a person's risk of developing severe dengue. Urbanization, particularly when unplanned, plays a major role in dengue transmission. Factors such as high population density, frequent human movement, inconsistent access to clean water, and common water storage practices all contribute to the spread of the disease. The risk of dengue within a community is also influenced by people's knowledge, attitudes, and behaviours related to the disease. Practices like water storage, keeping indoor plants, and using protective measures against mosquito bites directly impact exposure. Community participation in routine mosquito control and surveillance efforts can significantly strengthen resilience against outbreaks. Mosquito vectors may also adapt to changing environments and climates, making dengue transmission more unpredictable. The relationship between the dengue virus, its host, and the surrounding environment is constantly evolving. As climate change, rapid urbanization, and increased human migration continue to shape tropical and subtropical regions, the risks and patterns of dengue may shift over time.[7]

From January to April 2024, the CFR varied from 0% in Nepal to 1.09% in Bangladesh. However, the case definition used varies across countries, with some reporting only (lab-confirmed) hospitalised cases (compared to others reporting probable cases from communities), which leads to a higher case fatality rate among those hospitalised or severe dengue cases. Indonesia experienced a surge in dengue incidence in 2024, with 88,593 confirmed cases and 621 deaths as of 30 April 2024 – approximately three times higher than the same period in 2023.[6]

Vitamin D, also known as calciferol, is a fat-soluble vitamin found naturally in some foods, available as a supplement, and also, synthesized in the skin when exposed to ultraviolet (UV) sunlight.[8]

An essential component of the body's innate immune system is vitamin D. It helps attract key immune cells, including monocytes, T cells, and neutrophils, to areas where they are needed. Additionally, vitamin D influences the type of immune response the body produces, shifting it toward a Th2-type response. This means it increases levels of IL-4, IL-5, and IL-10, which are anti-inflammatory cytokines while reducing the production of pro-inflammatory cytokines such as IL-2, IFN-γ, and TNF-α. The active form of vitamin D, known as 1,25-dihydroxyvitamin D3, is derived from its precursor 25-hydroxyvitamin D, and plays an important role in regulating immune function. It helps suppress Th17 cells, which produce IL-17 and IL-22 and promotes the activity of CD4+/CD25+ regulatory T cells, which help keep the immune system in balance. Furthermore, vitamin D influences how T cells move throughout the body, particularly guiding them to the skin.[9] Levels of vitamin D may be a helpful prognostic indicator for forecasting the course of an illness.[10] A supplement of 4000 IU/day of vitamin D may represent an adequate dose to control dengue progression and DENV replication.[11]

Review question

Is there an association between Vitamin D serostatus and Dengue Severity?

Method

PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2020 standards will be followed during the review, which has been filed in PROSPERO

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(Registration ID: CRD420251003676).

Search Strategy

The studies will be identified from peer-reviewed literature. To identify peer-reviewed literature, "Dengue" and "Vitamin D" will be combined with synonyms to restrict the search to Dengue and measure its association with Vitamin D. The search timeline was restricted, from January 2000 to February 2025, no geographical restrictions were considered. Studies published in languages other than English and research articles that have not undergone peer review, such as thesis, conference proceedings, review articles, and letters to the editor/correspondence, will be excluded. The following databases would be used: MEDLINE (PubMed & PubMed Central), Scopus, and Web of Science. The first ten pages of Google Scholar, an academic search engine, will be searched.

Search terms

Using Boolean operators, search phrases were created and concatenated in the following ways to improve the likelihood that the results would be all-inclusive:

The search terms used were: "Dengue", "Dengue fever", "Dengue Severity" and "Vitamin D", "25-Hdroxycholicalciferol", or "Calcitriol". An advanced search would be undertaken by combining the search terms, (Dengue * OR Dengue fever OR Dengue Severity) AND (Vitamin D* OR Calciferol OR 25-Hydroxycholicalciferol).

Search detail

Scopus

(TITLE-ABS-KEY(Dengue) OR TITLE-ABS-KEY(Dengue fever) OR TITLE-ABS-KEY(Dengue severity) AND TITLE-ABS-KEY(Vitamin D) OR TITLE-ABS-KEY(Calciferol) OR TITLE-ABS-KEY(25-Hydroxycholicalciferol)) AND PUBYEAR > 2000 AND PUBYEAR <2026.

Medline

PubMed

(((((Dengue[Title/Abstract]) OR (Dengue fever[Title/Abstract])) OR (Dengue Severity[Title/Abstract])) AND (Vitamin D[Title/Abstract])) OR (Calciferol[Title/Abstract])) OR (25-Hydroxycholicalciferol[Title/Abstract])

PubMed Central

((((((Dengue[Title]) OR Dengue fever[Title]) OR Dengue Severity[Title]) OR Break-bone fever[Title]) AND Vitamin D[Title]) OR Calciferol[Title]) OR 25-Hydroxycholicalciferol[Title]

Web of Science

(TI=(Dengue) OR TI=(Dengue fever) OR TI=(Dengue Severity)) AND (TI=(Vitamin D) OR TI=(Calciferol) OR TI=(25-Hydroxycholicalciferol))

Eligibility criteria for study selection

Inclusion criteria

- **Primary:** Observational studies (e.g., cohort, case-control, cross-sectional), Randomized control trials assessing the association between serum Vitamin D levels and dengue severity.
- **Secondary:** Studies reporting relevant outcomes, including clinical severity and laboratory markers of dengue.

Exclusion criteria

Studies based on genetics and molecular basis, non-human studies, reviews, conference abstracts, and studies with inadequate data on Vitamin D levels or dengue severity.

Studies which are not in the English language.

Study selection

Two independent reviewers (Reviewer 1 and Reviewer 2) will screen the titles and abstracts of studies based on predefined eligibility criteria to determine their initial inclusion. Studies that meet these criteria will then undergo a full-text review for further evaluation. To ensure an unbiased selection process, both reviewers will be blinded to each other's decisions until the initial screening is complete. In cases where there are discrepancies in their assessments, they will first attempt to resolve them through discussion. If they are unable to reach an agreement, a senior third reviewer will step in to make the final decision. The study selection process will be conducted using Rayyan QCRI, a software designed for systematic reviews, ensuring that all inclusion and exclusion decisions are systematically recorded and tracked.

Inclusion Criteria (Relevant Contexts)

This study will include research from both dengue-endemic and non-endemic regions to provide a comprehensive understanding of the disease. It will cover studies conducted on both hospitalized and non-hospitalized dengue patients, spanning primary care, secondary care, and tertiary hospital settings. Additionally, studies involving patients in intensive care units (ICUs) will be included if they contain relevant data on Vitamin D levels. The study population will encompass all age groups, with a focus on research that assesses Vitamin D levels in dengue patients diagnosed through clinical or laboratory criteria. To ensure a broad and up-to-date analysis, studies published between January 2000 and February 2025 will be considered.

Exclusion Criteria (Contexts NOT Eligible)

This study will exclude research conducted solely on healthy individuals, as well as studies focusing exclusively on populations with severe comorbidities—such as chronic kidney disease, HIV/AIDS, or advanced liver disease—that

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could independently impact Vitamin D metabolism and interfere with the results. Additionally, studies that do not classify dengue severity according to the WHO 1997 or 2009 guidelines or fail to measure Vitamin D levels will not be considered.

The number of studies retrieved and the number chosen based on the title and/or abstract will be noted, along with the search terms used in each database. The spreadsheet containing peer-reviewed literature will have a few chosen research, along with an extra column designating the publication source. The selection of study included in the review will be based on the following revised PRISMA diagram.[12]

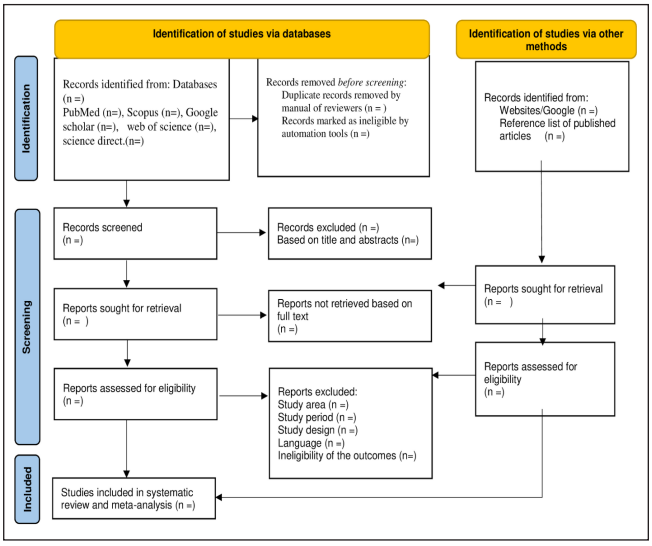

Figure 1.PRISMA 2020 Flow Diagram

Data Extraction and Collection

Data Extraction Methodology

Two impartial reviewers will use a standardized data extraction form to perform the data extraction. To ensure accuracy and completeness, a third reviewer will cross-check the extracted data. Any discrepancies will be resolved through discussion, and if needed, a senior reviewer will be consulted for a final decision.

Data to Be Extracted

For each included study, key information will be systematically collected. General study details such as the title, authors, year of publication, study design (observational or randomized controlled trial), and study setting will be recorded. Population characteristics will include the total sample size, age group distribution (children, adults, or elderly), gender breakdown, and the method used for dengue diagnosis (RT-PCR, NS1 antigen, or serology). Data on Vitamin D status will cover mean and median serum 25(OH)D levels (reported in ng/mL or nmol/L), classification of Vitamin D levels as deficient, insufficient, or sufficient, and any information on Vitamin D supplementation if available. To assess dengue severity, studies will be categorized according to the WHO 1997 and 2009 classification criteria. Microsoft Excel will be used for structured data entry and management throughout the extraction process.

Approach for Handling Missing Information

If essential data is missing from a study, the authors will be contacted via email to request additional details. When feasible, standard formulas will be applied to estimate missing values, such as converting standard deviations from confidence intervals. To ensure the reliability of the analysis, studies with substantial missing data may either be excluded or analyzed separately through a sensitivity analysis to assess their impact on the overall findings.

Data Synthesis

Meta-analysis in Microsoft Excel with MetaXL add-in will be carried out. Based on the type of measurement scale of the independent (Vitamin D in continuous or nominal scale) variable, appropriate statistical methods as per MetaXL guideline will be employed to estimate pooled statistical parameter. Studies that has measured effect measures, appropriate pooled analysis will be carried out only if ≥ 2 similar studies included in the study. Heterogeneity will be assessed using I^2 and Cochrane Q statistics. A Random effect or IV heterogeneity model will be used based on the heterogeneity of the studies. A forest plot will be used to identify the outliers. Sensitivity analysis will be carried out in order to explore the impact of different decisions (inclusion or exclusion) on results and to address the outliers.

Table 1.Data Extraction Table for Characteristics to be Included for Meta-Analysis

1st Author (Publication Year)	Dengue Type (Based on WHO 1997 or WHO 2009 Classification)	Sample Size (Total & Sub_Group: Adult Child Male Female)	Vitamin D Status (N if Vit D status expressed as a category variable i.eDeficient, Insufficient, Optimal) (Mean or Median if Vit D status is expressed in continuous level)

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Assessment of Methodological Quality

The quality of all observational studies will be assessed using the Joanna Briggs Institute (JBI) tool,[13] ROB[2] tool would be used to assess the quality of studies pertaining to Randomized control trials.[14] At least two independent reviewers will evaluate the data, and any discrepancies in their assessments will be resolved through a structured process. If any necessary information is unclear or missing from the published studies, the study investigators will be contacted to provide additional details.

Reporting Bias Assessment

Bias risk resulting from missing data will be evaluated. A funnel plot test will be used to gauge the degree of publishing bias.

Certainty Assessment

To assess the certainty (or confidence) in the bodies of evidence produced by the review, the GRADE (Grading of Recommendations, Assessment, Development, and Evaluations) approach will be used. This method evaluates the certainty of evidence based on the following five domains:

- **Risk of Bias:** The quality of individual studies will be assessed, considering factors such as study design, allocation concealment, blinding, and completeness of outcome data.
- **Inconsistency:** The consistency of results across studies will be examined. Large variations in effect estimates without a clear explanation may lower certainty.
- **Indirectness:** The applicability of the evidence to the research question will be evaluated. If the population, intervention, comparator, or outcomes differ significantly from those of interest, the certainty may be reduced.
- **Imprecision:** The confidence intervals and sample sizes of included studies will be assessed. Wide confidence intervals or small sample sizes may indicate lower certainty.
- **Publication Bias:** The likelihood of selective publication will be considered, using tools such as funnel plots and statistical tests when sufficient studies are available.

Based on these assessments, the overall certainty of the evidence for each outcome will be rated as high, moderate, low, or very low. Any downgrading or upgrading of the certainty level will be transparently justified in the review.

Plans for Updating the Review

Versions of this Systematic Review and Meta-Analysis technique will be available for revision or expansion as needed.

Ethical Approval and Consent to Participate

This is not applicable as no primary data would be collected.

Discussion

There has been no previous Systematic review and meta-analysis on "Association between Vitamin D and Dengue Severity." This study will help resolve the prevailing ambiguity to a certain extent and would also be an additional highlight in determining the role of Vitamin D in the progression of Dengue fever, which is a re-emerging neglected tropical disease. Vitamin D insufficiency or deficiency (VDD) has been identified as a prevalent condition in the general population, and also it can result in other conditions like obesity, diabetes, hypertension, depression, and other conditions .[15]

The systematic review and meta-analysis will be done according to the PRISMA 2020 guidelines.[12] The review will include only peer-reviewed articles with no Geographical restrictions. The potential limitation of this review is that it would include articles that have been published in the English language only.

Conclusion

Dengue although a self-limiting disease, is life-threatening especially in tropical regions, with the advent of urbanization and globalization the rise in the prevalence of this disease is a pace faster than earlier. The introduction of a sedentary lifestyle has come at the cost of human health, wherein limited or no exposure to Vitamin D is a major contributing factor in the deficiency of Vitamin D. Therefore, the results from this study will help researchers and clinicians identify the importance of Vitamin D and its role in dengue severity.

Authors' Contributions

All the authors have contributed equally to drafting the manuscript. Two authors have been assigned the role of primary reviewers, any discrepancy between them would be resolved by a third reviewer or co-author. The other co-authors would assess the quality of the studies considered and publication bias.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process: None

Conflict of Interest: None

Source of Funding: None

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