

Review Article: Micronutrients and Dengue

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Abstract. Dengue virus infection is the most widespread mosquito-borne viral infection in humans and has emerged as a serious global health challenge. In the absence of effective treatment and vaccine, host factors including nutritional status, which may alter disease progression, need investigation. The interplay between nutrition and other infections is well-established, and modulation of nutritional status often presents a simple low-cost method of interrupting transmission, reducing susceptibility, and/or ameliorating disease severity. This review examines the evidence on the role of micronutrients in dengue virus infection. We found critical issues and often inconsistent results across studies; this finding along with the lack of sufficient literature in this field have limited our ability to make any recommendations. However, vitamins D and E have shown promise in small supplementation trials. In summary, the role of micronutrients in dengue virus infection is an exciting research area and needs to be examined in well-designed studies with larger samples.

INTRODUCTION

Dengue virus (DENV), a member of the *Flaviviridae* family, causes the most widespread mosquito-borne viral infection in humans around the world today. The *Flaviviridae* family is comprised of over 70 viruses,¹ in which DENV is a single positive-stranded RNA virus transmitted by the mosquitoes *Aedes aegypti* and *Ae. albopictus*.² There are four serotypes of DENV that cause infection. DENV infection can result in either asymptomatic infection or mild undifferentiated fever, or it can take one of three clinical manifestations in humans: dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). About one-half of DENV infections exist as asymptomatic, and some exist as undifferentiated fever (in which the patient experiences fever and mild symptoms, but the source of infection is not diagnosed as DENV). The three clinical manifestations of the disease differ in the severity of their symptoms, with the flu-like DF being the least severe and DSS being the most severe. In most cases, the mild febrile DF is not fatal; the infection turning into DHF or DSS, however, can be life-threatening and causes mortality in many cases. Patients with DHF and DSS have been found to have 100- to 1,000-fold higher virus titers than patients with DF from the initial phase of infection.³ Overall, DENV infection has been found to be more severe in children than adults.⁴

Over the past few years, DENV infection has rapidly emerged as a global threat to human health, with its rate of occurrence, geographic distribution, and clinical severity increasing its global burden significantly. DENV infection has been reported from over 100 countries, in which approximately 2.5 billion people live in endemic regions; the current estimate of the number of annual dengue cases is 100 million for DF and 250,000 for DHF, with a total of 25,000 deaths per year.⁵ In fact, these numbers may underestimate the true burden of dengue by almost three times, which was suggested by a recent publication.⁶

The pathophysiology of DENV in the body and the host's immune response are not completely understood. Major dis-

ease manifestations in the body include capillary leak syndrome (plasma leakage caused by endothelial cell dysfunction, which is specific to DHF), thrombocytopenia (which is seen in all forms of DENV infection, but very severe values are specific to DHF), leukopenia, and hemorrhagic tendencies.⁷ It is known that the major viral envelope (E) glycoprotein in the virus assists its binding to host cells,⁸ after which the virus enters the cell and viral replication occurs. Data suggest that monocytes are the primary target.⁹ Infected monocytes induce the production of interferon- α (IFN- α) and IFN- β .¹⁰ E, precursor membrane protein (pre-M), and nonstructural protein 1 (NS1) are the major proteins on DENV that are targeted by antibodies as part of the host immune response. Studies show that DENV-specific CD4+ and CD8+ T lymphocytes attack infected cells and release IFN- γ , tumor necrosis factor- α (TNF- α), and lymphotoxin.⁷ Primary infection induces lifelong immunity in the individual to that particular serotype but not to secondary infection by a different serotype.

Host nutritional status is a strong predictor of immunity¹¹; in fact, malnutrition is the most common cause of immunodeficiency worldwide,¹² estimated to cause about 50% of childhood deaths and a significant fraction of deaths from infectious diseases in developing countries.¹³ A properly functioning immune system requires an adequate supply of micronutrients to both prevent damage of cells participating in the innate immune response and restore tissues damaged from the host defense against the infectious agents.^{14–20} Earlier studies have, contradictorily, found that malnourished children are less likely to develop DHF/DSS compared with well-nourished children, but recent studies have not supported this finding.²¹

Overall, the association between nutritional status and risk of DENV infection remains unclear. There is no evidence to suggest that nutritional status could interrupt transmission or alter susceptibility to infection after the bite of a dengue-infected mosquito; however, host nutritional status or micronutrient supplementation as adjuvant therapy could lower the probability of progressing from DENV infection to overt/severe forms of disease or reduce disease severity in patients. Although there is no replacement for an effective vaccine for the virus, modulation of nutritional status or micronutrient supplementation can act as supportive therapy to help patients suffering from dengue infection and is the primary focus of the literature reviewed here in this manuscript.

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METHODS

The literature reviewed was identified using various searches in PubMed, WorldCat, and Google Scholar as well as citation tracking on original research articles. The searches done were in the form of “dengue and...,” of which the broader search terms used to initially identify the specific micronutrients for the review were nutrition, micronutrients, and vitamins. More specific search terms pertaining to the individual sections included vitamin A, retinol, retinoic acid, α -carotene, β -carotene, γ -carotene, xanthophyll, antioxidants, zinc, iron, anemia, vitamin C, vitamin E, vitamin D, vitamin B, thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, folic acid, folate, cobalamin, chromium, magnesium, DF, DHF, DSS, and supplementation. Articles were screened based on titles and abstracts, and all relevant articles were retrieved. Bibliographies of all retrieved articles were scanned for additional relevant articles. Because of the limited literature available on this subject, all laboratory, observational, and intervention studies found on dengue and the specific micronutrients discussed were chosen for inclusion in this review (a total of 15 articles).

All included studies are discussed below by the specific nutrient evaluated, highlighting the rationale for each nutrient followed by the evidence from observational studies and randomized trials where available.

RESULTS

Vitamin D. *Why vitamin D?* Vitamin D is known to play an essential role in the immune system, and vitamin D deficiency has long been associated with autoimmune diseases as well as increased susceptibility to viral infections.²² Vitamin D has been shown to promote both innate and adaptive immunity through a number of mechanisms,²³ such as T-cell activation and monocyte differentiation. Many additional cells of the immune system (including B cells, monocytes, and dendritic cells) also respond to the immunomodulatory effects²⁴ of vitamin D through the vitamin D receptor (VDR) expressed on their cell surface. Vitamin D binding to the VDR, in turn, activates vitamin D-responsive genes in the body, many of which induce a number of pathogen-fighting mechanisms. Vitamin D supplementation also has had some success in helping to treat other viral infections, such as influenza.²⁵

A recent laboratory study conducted in Mexico investigated the effect of treatment with 1,25-dihydroxyvitamin D₃ on two types of human cell lines (hepatic Huh-7 and monocytic U937) infected with DENV-4. Puerta-Guardo and others²⁶ found that exposure to 1,25-dihydroxy vitamin D₃ significantly reduced the number of infected cells, particularly in monocytic cells, and lowered the production of proinflammatory cytokines (TNF- α , interleukin-6 [IL-6], IL-12p70, and IL-1 β). The highest concentration of 1,25-dihydroxy vitamin D₃ (10 μ M) induced the greatest reduction in percentage of infected cells, suggesting a correlation between vitamin D₃ dose and inhibition of DENV infection.

In monocytic cells, the primary target for DENV, this effect is believed to be because of the interference of vitamin D₃ with the activation of several host cell signaling pathways that are essential for DENV survival and replication. It has been shown that vitamin D₃ down-regulates the Toll-like receptors (TLRs) that activate the nuclear factor- κ B (NF- κ B)/RelA

pathway, which reduces the phosphorylation of mitogen-activated protein kinases (MAPKs) p38 and p42/44 as well as the production of TNF- α and IL-6.²⁷ The activation of MAPKs c-Jun N-terminal kinase (JNK) and p38 pathways is necessary for the virus to successfully replicate and infect macrophages that release proinflammatory cytokines, which are associated with the disease manifestations of capillary leak syndrome and hemorrhagic tendencies.^{28,29} Therefore, down-regulation of this pathway by vitamin D₃ would result in the reduction of the numbers of infected cells and specific proinflammatory cytokines that were observed in this study as well as hypothetically, a reduction in clinical severity of the disease.

Evidence from observational studies. Multiple observational studies have also investigated the relationship between vitamin D and DENV infection in patients with dengue. A recent study in India compared the levels of vitamin D in patients with DF and DHF with those of healthy individuals. Alagarasu and others³⁰ found that patients with both DF and DHF had significantly higher 25-hydroxyvitamin D levels in their blood than the healthy controls ($P < 0.005$ and $P < 0.001$, respectively). Alagarasu and others³⁰ also found that patients with secondary DHF had significantly higher vitamin D concentrations than patients with secondary DF ($P < 0.05$). Alagarasu and others³⁰ speculated that this association might be related to the inducing effect of vitamin D on Fc γ -receptor expression (which enhances viral entry into cells, possibly leading to higher viral load in dengue cases with secondary infection and the development of DHF) or dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN), the primary receptor through which the virus enters immature dendritic cells.

In a similar observational study in Brazil, Albuquerque and others³¹ compared protein levels in the plasma of patients with severe DF with the protein levels of healthy individuals and found that a number of proteins increased or decreased in patients with severe DF; one of the proteins showing a significant increase in DF patients was vitamin D-binding protein.³¹ Because vitamin D-binding protein is the major plasma carrier of vitamin D, its increase associated with higher levels of vitamin D is expected.

In another study in Pune, India, Alagarasu and others³² assessed the frequencies of various vitamin D-receptor gene polymorphisms in DF and DHF patients as well as healthy controls. All dengue patients and DF patients were found to have a significantly lower frequency of the haplotype G-C-T (corrected P -value [Pc] = 0.0135) and a higher frequency of the haplotype G-A-T (Pc = 0.000085) compared with healthy controls. The results suggest that the 3' untranslated region (UTR) haplotypes of the VDR gene are differentially associated with risk of symptomatic dengue requiring hospitalization.

A study in Vietnam among DHF grade III patients, DHF grade IV patients, and healthy controls found that the t allele of a variant at position 352 of the VDR gene was associated with resistance to severe dengue ($P = 0.03$); the allele frequencies of the t allele found were 3.4%, 1.9%, and 0% for the controls, DHF grade III cases, and DHF grade IV cases, respectively.³³

Evidence from trials. A small trial conducted in Mexico examined the effects of calcium and vitamin D supplementation among a total of five DF patients.³⁴ In addition to receiving the standard treatment of electrolytic solutions and 500 mg paracetamol (acetaminophen) every 12 hours, the participants received a specific course of calcium carbonate

plus vitamin D3 supplementation (more details in Table 1). Sánchez-Valdéz and others³⁴ observed a significant increase in platelet count in all five of the patients; the average platelet count changed from $136,000 \pm 69,508$ cells/mm³ before treatment to $179,600 \pm 56,584$ cells/mm³ after treatment. Sánchez-Valdéz and others³⁴ also observed a significant improvement in the overall clinical condition of the patients as well as reduction in the duration of signs and symptoms of the infection. Sánchez-Valdéz and others³⁴ suggest that the supplementation may possibly restore free Ca²⁺ quicker, leading to the reduced thrombocytopenia observed.

Zinc. *Why zinc?* Similar to vitamin D, zinc is also very important for immune function, and deficiency in zinc has been associated with decreased resistance to viral infection.³⁵ Affecting a number of immune cells and functions, zinc specifically influences lymphocyte maturation, cytokine production, and generation of free radicals while maintaining normal macrophage and natural killer (NK) cell activity in the immune response³⁶; it also plays a role in T-cell and neutrophil activity as well as B-cell development. Zinc supplementation has also been found to reduce mortality from diarrhea and pneumonia³⁷ and has been shown to be beneficial in preventing respiratory infection.³⁸

In a laboratory study conducted in Malaysia, Shafee and AbuBakar³⁹ studied the effect of different concentrations of Zn²⁺ on apoptosis of DENV-2-infected Vero cells and found that they quickened apoptosis of the infected cells. The acceleration of apoptosis by zinc could represent a mechanism through which zinc supplementation may help host limit viral infection. Another study using human neuroblastoma cells showed that treatments of DENV-2-infected cells with ZnSO₄ at low concentrations (< 20 μM) resulted in dose-dependent protection of the infected cells, whereas at higher concentrations, Zn²⁺ became toxic.⁴⁰

Evidence from observational studies. An observational study in Indonesia investigated serum zinc levels in children with DF, DHF, and DSS. Yuliana and others⁴¹ found that a decrease in zinc levels associated with increasing severity of DENV infection. However, in a similar observational study in Indonesia, Widagdo⁴² found no significant association between clinical severity of DHF and blood zinc levels.

Vitamin A. *Why vitamin A?* Vitamin A, one of the most commonly studied nutrients in relation to immunity, is known to be a central regulator of the immune system; vitamin A deficiency has been shown in many studies to impair both humoral and cell-mediated immunity as well as the integrity of epithelial tissues of the eyes, lungs, and gut,⁴³ all of which lead to an increased susceptibility to pathogens and infectious diseases. Specifically, vitamin A affects the activity of macrophages and the number and activity of NK cells as well as lymphocyte functions, such as B-cell proliferation and T-cell activation.⁴⁴ Vitamin A supplementation has been found to have a significant impact on preventing morbidity and mortality in a number of infectious diseases in developing countries.⁴⁵ Studies show that vitamin A supplementation decreases disease severity and risk of death in malaria⁴⁶ and reduces mortality in measles.⁴⁷

Evidence from observational studies. There has been very little research on the association between vitamin A and DENV infection in humans. In an observational study conducted in Guatemala, Klassen and others⁴⁸ compared the plasma concentrations of circulating antioxidant nutrients

(including retinol and β-carotene) of patients with DF with those of healthy individuals. Klassen and others⁴⁸ found an association between dengue and lower levels of both retinol and β-carotene.

Iron. *Why iron?* The need for iron for proper immune function stems from its role in promoting the growth and differentiation of various immune cells; specifically, iron deficiency has been found to decrease mitogen responsiveness, NK cell activity, lymphocyte bactericidal activity, and neutrophil phagocytic activity while influencing cytokine activity in every stage of the immune response to infection.⁴⁹

Evidence from observational studies. There are no studies, to the best of our knowledge, that have comprehensively assessed iron status or examined the effects of iron supplementation in the context of DENV infection. In one observational study in Thailand, Chaiyaratana and others⁵⁰ compared serum ferritin levels (one of the indicators of iron status) in children with DF and DHF during the infection and at follow-up 2–4 weeks after discharge from the hospital. Chaiyaratana and others⁵⁰ found that serum ferritin levels were higher in both DF and DHF patients during the infection than at follow-up, with DHF patients displaying higher ferritin levels than DF patients throughout the course of the illness. These results are not surprising given that ferritin is an acute-phase reactant. The World Health Organization (WHO) recommends that comprehensive iron status assessment should include measurement of hemoglobin, serum ferritin, serum transferrin receptor, and at least one inflammatory biomarker (C-reactive protein or α-1 acid glycoprotein).⁵¹

Chromium. *Why chromium?* Chromium (an essential trace mineral) has been known for its effects on the regulation of blood sugar by promoting the action of insulin, and recently, it has been discovered to affect the immune response by influencing T and B lymphocytes, antigen-presenting cells (such as macrophages), and cytokine production. Chromium supplementation is known to increase immune function in animals, possibly by reducing serum cortisol levels. Chromium supplementation has been shown to exhibit very complex effects; high doses and extended exposure can make hexavalent chromium cytotoxic to the body⁵² by inhibiting many cellular processes and mutating genes important to the immune response. In addition, exposure to chromium from the environment has been reported to cause many adverse health effects.⁵³ Therefore, chromium supplementation, if ever used to benefit infections or diseases, must have a very precise dose.

A few studies on mice suggest a possible association between chromium and DENV infection. In a study in India, Shrivastava and others⁵⁴ studied the effect of hexavalent chromium on DENV infection in mice. Shrivastava and others⁵⁴ first exposed the experimental group to Cr (VI) and then infected both groups with DENV, and they found that exposure to Cr (VI) significantly helped minimize the effects of infection.⁵⁴ Specifically, the reduction in the total platelet count after DENV infection of mice exposed to Cr (VI) was significantly less than that in the control group mice.

In a similar 2005 study conducted by some of the same researchers, the spleens of the male mice were studied and found to reduce significantly in weight as a result of Cr (VI) exposure.⁵⁵ Shrivastava and others⁵⁵ observed a reduction in the weight of the spleen as a result of DENV infection. This reduction was significantly greater with Cr (VI) exposure and

TABLE 1
Summary of research studies

Study	Sample	Dengue case definition	Exposure	Results
Laboratory Mexico ²⁶	2 × 10 ⁵ Huh-7 or U937 DENV-infected cells	–	VD3 at concentrations of 0, 0.001, 0.01, 0.1, 1, and 10 μM	VD3 significantly reduced percentage of infected cells and TNF-α, IL-1β, IL-6, and IL-12p70 concentrations
Malaysia ³⁹	C6/36 mosquito and Vero DENV-infected cells (African green monkey kidney)	–	Ca ²⁺ , Mg ²⁺ , Mn ²⁺ , or Zn ²⁺ at concentrations of 0.1, 0.5, 1.0, 2.5, and 5.0 mM	Higher Zn ²⁺ concentrations significantly increased rate of apoptosis (mechanism thought to limit virus infection)
India ⁵⁴	Swiss mice (25–30 g, ages 6–8 weeks); N = 72 experimental group, N = 24 control group	–	250 ppm chromium (VI; 14.8 mg/kg per day per mouse)	Significantly less reduction in platelet count (reduced thrombocytopenia) in infected treated mice
India ⁵⁵	Swiss mice (25–30 g, ages 6–8 weeks)	–	250 ppm chromium (VI; 14.8 mg/kg per day per mouse)	Significantly greater reduction in spleen weight, higher cytotoxic activity, and greater reduction in phagocytic activity in infected treated mice
India ⁵⁸	Swiss mice (25–30 g, ages 6–8 weeks); N = 18 in two experimental groups, N = 18 control group	–	100 mg/L CrP versus 250 mg/L CrP	Significantly less reduction in platelet count (reduced thrombocytopenia) in infected treated mice
Observational India ³⁰	N = 48 DF cases (42.5% primary cases), N = 45 DHF cases (18.6% primary cases), N = 20 healthy controls	DF and DHF cases defined by WHO criteria (1999)	–	DF and DHF patients had significantly higher 25(OH)D concentrations compared with healthy controls; secondary DHF patients had significantly higher 25(OH)D concentrations than secondary DF patients
Brazil ³¹	N = 13 severe DF cases, N = 13 healthy controls	Confirmed dengue infection by antidengue ELISA IgM, serotype-specific RT-PCR, or virus isolation. Severe DF cases were defined by the following criteria: confirmed dengue cases with severe thrombocytopenia ($< 50,000$ platelets/mm ³), hypotension (postural hypotension with a decrease in systolic arterial pressure of 20 mmHg in supine position or a systolic arterial pressure < 90 mmHg), plasma leakage (either hemoconcentration fluctuation of packed cell volumes $\geq 20\%$ during the course of illness and recovery or clinical signs of plasma leakage, such as pleural effusion), and/or severe hemorrhagic manifestations. All patients were admitted to the hospital < 48 hours before the time of study and were from same region of a recent outbreak of DENV-3	–	DF patients had increased DBP levels and up-regulated DBP expression
India ³²	N = 85 DF cases, N = 29 DHF cases, N = 106 healthy controls	Confirmed infection by dengue-specific IgM ELISA and/or RT-PCR. DHF cases fulfilled at least two of the WHO criteria (1999)	–	Identified vitamin D receptor gene polymorphisms, which increase susceptibility to infection
Vietnam ³³	N = 315 DHF grade III cases, N = 37 DHF grade IV cases, N = 251 healthy controls	DHF cases and severity defined by WHO criteria (1997)	–	Vitamin D receptor gene (r allele of a variant at position 352) was associated with resistance to severe dengue

(continued)

TABLE 1
Continued

Study	Sample	Dengue case definition	Exposure	Results
Indonesia ⁴¹	N = 20 DF cases, N = 20 DHF cases, N = 20 DSS cases (all children < 14 years old)	Confirmed infection by serologic test using rapid test dengue blot. DF, DHF, and DSS cases were defined by WHO criteria (1997)	–	Lower serum Zn concentrations were significantly associated with increased severity of infection
Indonesia ⁴²	N = 45 DHF (children; N = 29 grade I, N = 12 grade II, N = 2 grade III, N = 2 grade IV)	Confirmed infection by serologic test. DHF cases and severity defined by WHO criteria (1999)	–	No significant associations between serum Zn concentrations and severity of infection
Guatemala ⁴⁸	N = 10 DF cases (18–68 years old), N = 12 healthy controls	Confirmed infection by dengue-specific IgM ELISA or virus isolation	–	Low retinol and β -carotene levels were significantly associated with infection
Thailand ⁵⁰	N = 44 DF cases, N = 133 DHF cases (100 male, 77 female; 4–16 years old; 10 primary infection, 177 secondary infection)	Confirmed infection by dengue-specific IgM and IgG ELISA DHF cases defined by WHO criteria (1997)	–	Serum ferritin levels were significantly higher during infection than at follow-up (DHF greater than DF)
Interventions/trials				
Mexico ³⁴	N = 5 DF cases (3 male, 2 female; 18–59 years old)	Positive for tourniquet test (Rumpel–Leede capillary fragility test); fulfilled at least four of clinical criteria for DF case definition	200 IU vitamin D2 and 600 mg calcium carbonate: one time per 8 hours for the first 3 days, one time per 12 hours for next 3 days, and one time per day until patients were free of dengue symptoms (≥ 3 days)	Increased platelet count, improved overall clinical condition, reduced duration of negative signs and symptoms, and reduced recovery time
India ⁶³	N = 66 DF patients	DF cases defined by WHO criteria (2009)	400 mg vitamin E per day	Faster increase in platelet count (combating thrombocytopenia)

DBP = vitamin D-binding protein; ELISA = enzyme-linked immunosorbent assay; Ig = immunoglobulin; IU = international unit; RT-PCR = reverse transcription polymerase chain reaction; VD3 = 1,25-dihydroxyvitamin D3.

greatest with the longest duration of exposure. In addition, Shrivastava and others⁵⁵ also observed a significantly higher cytotoxic activity in the spleen homogenates obtained from the mice exposed to Cr (VI) for 9 weeks as opposed to those not exposed to Cr (VI) ($P < 0.001$) as well as a higher reduction in phagocytic activity in the splenic macrophages. The mechanism of action of this Cr (VI)-induced cytotoxicity is not clearly understood, but it is known that immune cells work to reduce Cr (VI) to the less toxic Cr (III) on entrance into the body.⁵⁶ Studies have shown that, during this process, Cr (VI) induces the tumor suppressor gene p53 and enhances the production of reactive oxygen species (specifically superoxide ion, hydroxyl radicals, and hydrogen peroxide), inducing oxidative stress and causing damage to genomic DNA and biological macromolecules.⁵² Studies have also shown chromium to have a dose-dependent effect on alveolar macrophages, activating with smaller doses and inhibiting with higher doses.⁵⁷

In another laboratory study by the same team, Shrivastava and others⁵⁸ exposed mice to a different type of chromium, chromium picolinate (CrP), to investigate its effects on DENV infection and found that it also significantly increased platelet counts.⁵⁸

Vitamin E. *Why vitamin E?* Immune function has been found to be especially sensitive to changes in vitamin E status; even marginal vitamin E deficiency prevents the immune system from exhibiting a proper response to infection.⁵⁹

Importantly, the antioxidant properties of vitamin E protect immune cell membranes from oxidative damage. Vitamin E supplementation has been reported to enhance both humoral- and cell-mediated immune responses and resistance to infection⁶⁰ in a number of human studies. It has been shown to enhance immunity in elderly populations.⁶¹ Specifically, vitamin E enhances T-cell differentiation, helper T-cell and NK cell activity, lymphocyte proliferation, and macrophage function.⁶²

Evidence from trials. In a recent trial conducted in India, Vaish and others⁶³ administered vitamin E supplements to patients with DF to study the effects of vitamin E on thrombocytopenia. All patients had initial platelet counts determined to be between $10 \times 10^3/\text{mm}^3$ and $100 \times 10^3/\text{mm}^3$. Divided into two groups of 33 patients each, Vaish and others⁶³ gave one group 400 mg oral vitamin E supplementation in addition to the standard treatment, whereas the other group received only standard treatment. Vaish and others⁶³ followed up both groups for 7 days and determined platelet counts at specific intervals, and they discovered a significant increase in platelet count in the subjects exposed to vitamin E compared with those who were not.⁶³ Vaish and others⁶³ also found that the number of cases with thrombocytopenia severe enough to require platelet transfusion was lower in the group exposed to vitamin E (2 of 33; 6.06%) compared with the group not exposed to vitamin E (5 of 33; 15.15%). Although there is little known regarding the pathogenesis of thrombocytopenia in DENV infection, there is evidence that

it might be caused by increased oxidative stress⁶⁴; Klassen and others⁶⁴ suggest that the antioxidant properties of vitamin E might act to reduce oxidative stress and thus, restore platelet levels.

DISCUSSION

The lack of sufficient literature on the subject of micronutrients and DENV infection provides us with very few studies to make many specific recommendations. Most studies are observational with small sample sizes, and they include a limited assessment of potential confounding variables, such as inflammation, when assessing iron, vitamin A, or zinc status. Furthermore, the results are often inconsistent across different studies. Only vitamins D and E have been examined in the context of supplementation trials, with no control group and only five patients in the vitamin D trial, although the results are encouraging. Additionally, there is limited information on the optimal time during the course of the illness that nutritional supplementation may be most beneficial.

Biologically, nutritional status and supplementation are known to modulate immune function and likely to affect both the risk of DENV infection and the course of the illness. The biological plausibility for each of the micronutrients is discussed above in Results. However, more research studies with sufficient sample size and comprehensive assessment of risk factors and confounders for DENV infection are urgently needed to shed more light on the exact mechanisms involved. For example, on one hand, vitamin D may contribute to dengue pathogenesis by altering the response of particular ILs and enhancing the expression of DENV entry receptors that promote viral entry into cells, which may explain why the observational studies reviewed above found elevated levels of vitamin D and vitamin D-binding protein in patients with dengue. On the other hand, vitamin D status is known to be associated with a lower risk of several other infections in many larger studies. High-dose vitamin D supplementation and hydroxychloroquine have also been shown to successfully treat immune thrombocytopenia in two cases (believed to be by the down-regulation of CD4+ T cells and up-regulation of T-regulatory cells by vitamin D3, leading to restored platelet levels).⁶⁵ Similarly, leukopenia has long been associated with vitamin D deficiency (believed to be because of the requirement of white blood cell activation by vitamin D binding to vitamin D receptors on their surface), which suggests another possible mechanism by which vitamin D supplementation can reduce clinical severity of DENV infection.

Smaller studies have found associations between vitamin D receptor polymorphisms and DENV infection. It is possible that defective vitamin D-receptor signaling (caused by certain polymorphisms) and thus, ineffective vitamin D response might result in increased TNF- α levels and decreased IL-10 levels, leading to DHF. However, a large genome-wide association study (GWAS) in Vietnam did not identify vitamin D receptor genes as being associated with DENV infection.⁶⁶ It is worth noting that this study only focused on DSS among children.

The trial with vitamin E supplementation provides exciting evidence that suggests its therapeutic potential for dengue patients with biological plausibility. The administration of vitamin E is safe and simple⁶³; nonetheless, additional investigation with larger trials is necessary to confirm this result. Because this study is limited to only DF patients, future stud-

ies should include DHF patients as well. Vitamin E has also been shown to mitigate oxidative stress and leukopenia in previous laboratory studies,⁶⁷ which suggests possible mechanisms by which vitamin E supplementation can reduce clinical severity of DENV infection.

The evidence reviewed on chromium suggests a potential benefit of chromium supplementation for dengue patients and an overview of its mechanism of action. Limitations of the studies on chromium, however, include that they were all tested in mice and thus, cannot be directly applied to humans and that all evidence came from the same research team. Additional studies on CrP, such as laboratory studies on human cell lines and observational studies investigating a possible chromium deficiency in dengue patients, should be conducted.

Future research should investigate possible associations of DENV infection with other vitamins and micronutrients in addition to the ones reviewed here, because they have proven to be beneficial in other infections. Multivitamin supplementation (including vitamins B, C, and E) has been shown to be beneficial for patients with human immunodeficiency virus (HIV)⁶⁸ and tuberculosis.^{69,70} Vitamin B₁₂ has been shown to decrease viral replication and increase sustained viral response rates in patients of hepatitis C.⁷¹ Similarly, vitamin C supplementation has been shown to be beneficial against respiratory tract infections and pneumonia,⁷² whereas selenium supplementation has been shown to reduce parasitemia and the induced organ damage in parasitic infections, such as *Trypanosoma*.⁷³

More trials need to be conducted with each of the specific micronutrients discussed in this review; vitamin D and vitamin E, in particular, both require larger observational studies followed by randomized trials to confirm the positive results shown in past studies, because they propose the strongest cases for a possible benefit for dengue patients among all of the micronutrients reviewed here. Future studies should ideally include subjects of all ages instead of being restricted to only a particular age group (such as children) and investigate all severities of DENV infection (DF, DHF, and DSS) instead of being limited to just one to be applicable to a greater number of people and disease cases. The timing of supplementation needs to be evaluated, and because many biomarkers of micronutrient status are affected by the inflammatory response, measurements should be accompanied by assessing levels of either C-reactive protein or α -1 acid glycoprotein to facilitate accurate interpretation.

Considering the potential of micronutrient supplements to represent low-cost and simple adjuncts to improve treatment success in patients with dengue, it is surprising that the scope of research in this area has been rather limited. Researchers should also evaluate the possibility of nutritional status being a predictor of acquisition of DENV infection in endemic areas.

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