

Low Concentrations of Serum Vitamin D in Patients with Dengue without Warning Signs

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ABSTRACT

Objective: The host immune response may be a key component in the development of complications of dengue fever. Vitamin D has been shown to function as a modulator of the inflammatory response. The aim of this study was to determine the possible association between serum levels of vitamin D (25-hydroxyvitamin D) and patients diagnosed with dengue without warning signs (DWWS).

Materials and Methods: The vitamin D (VD) serum concentrations of 41 patients with clinical symptoms of DWWS and either a positive IgM test against the virus or a positive NSI viral antigen were measured and compared to the same number of sex and age-matched healthy patients. Patients under vitamin supplementation were excluded from the study.

Results: Vitamin D concentrations were found to be significantly lower in dengue patients ($p < 0.050$), median value 18.09 mg/dL, as compared to healthy controls, 21.14 mg/dL, particularly in female patients. Additionally, when the prevalence of patients with DWWS was analyzed comparing quintiles of serum VD concentrations of all patients included in the study and using the VD cut points suggested by the Endocrine Society, such prevalence increased as the VD concentrations decreased.

Conclusion: The results of the present study suggests that patients with low concentrations of vitamin D might be at higher risk for the development of DWWS, particularly female patients.

Keywords

Vitamin D, Dengue, Dengue without warning signs, Immune modulator.

Introduction

Dengue fever is considered the world's most important arthropod-borne viral disease affecting humans in terms of morbidity and mortality and is considered by the World Health Organization (WHO) as a major global public health challenge in the tropic and sub-tropic nations, with the number of cases increasing approximately 6 fold from 2010 to 2016 and with 40% of the world population at risk [1]. The year 2019 saw an increment in dengue cases worldwide and Mexico was no exception with

41,317 reported cases in the entire country and 1,678 in the state of Oaxaca alone [2]. Dengue is caused by any of 4 serotypes of the dengue virus (DENV), a member of the genus *Flavivirus* of the family *Flaviviridae*, and is transmitted to humans by the bites of infected mosquitoes, *Aedes aegypti* and *Aedes albopictus*.

DENV infection in humans results in a spectrum of outcomes that range from asymptomatic infections to symptomatic conditions that can be classified in three groups according to WHO: a) dengue "without warning signs" (DWWS); b) dengue "with warning signs of severe infection" (DWWS); c) severe dengue (SD) [3]. DWWS includes individuals with fever and at least two of the following signs and symptoms: nausea/vomiting, rash, headaches, eye pain,

muscle aches, joint pain, leukopenia, or positivity at the tourniquet test; DWSI includes, in addition to signs and symptoms of DWWS, abdominal pain, persistent vomiting, ascites, pleural effusion, mucosal bleeding, lethargy or restlessness, hepatomegaly, and an increase in hematocrit paired with rapid decrease in platelet count; SD occurs when the infection leads to severe plasma leakage, massive bleeding, and multiple organ failures [4].

While the pathophysiology behind the complications of dengue are not fully understood, and although factors such as viral virulence and host genetics might play a role in the pathogenesis of dengue, the host immune response may be a key component in the development of such complications [5]. Preexisting antibodies to the virus due to a past infection with a different viral serotype are not protective and can lead to a phenomenon called antibody-dependent enhancement (ADE) of infection, which in case of dengue, can enhance DENV infection of human macrophages by promoting fusion of the virus particles within endosomes and inhibits the antiviral response [6]. Additionally, the unbalanced production of certain proinflammatory cytokines (cytokine storm) such as TNF- α , VEGF-A, IL-6, IL-8, IL-10, CCL2, and CXCL10 has also been related to the pathogenesis of the complications of dengue [7].

In addition to the traditionally known classic actions of vitamin D (VD) on the homeostasis of calcium and its impact on bone health, in recent years this prohormone has been found to influence different biological processes within the human body, including the modulation of the immune response, both innate and adaptive. Several studies have concluded an intimate relationship between vitamin D and the production of the antimicrobial peptide cathelicidin [8] as well as in the modulation of cytokine production by T lymphocytes [9] and the enhancement of the phagocytic capacity of macrophages by this vitamin [10]. Additionally, Giraldo et al have recently demonstrated a possible link between VD and dengue infection, showing that high concentrations of vitamin D are able to control viral replication inside macrophages, as well as to impact the production of pro and anti-inflammatory cytokines during this infection [11]. Furthermore, a small study performed in Pakistan has shown that treatment of dengue patients with high doses of VD decreases the risk of dengue complications [12].

Since vitamin D has been shown to modulate the immune response and it has been postulated that this vitamin can have an effect on the progression of dengue, the aim of this study was to determine the possible association between serum levels of vitamin D (25-hydroxyvitamin D) and patients diagnosed with dengue without warning signs (DWWS).

Materials and Methods

Patients

A total of 41 ambulant adult patients (25 women and 16 men) from the city of Oaxaca, Mexico with clinical symptoms of DWWS and either a positive IgM test against the virus or a positive NS1 viral antigen (SD Dengue Duo, Korea) and 41 healthy age and sex-matched controls with no clinical symptoms of dengue were

included in the study. Patients with warning signs of severe dengue infection and severe infections were excluded. In both groups, patients taking vitamin supplements, including vitamin D, were also excluded.

Laboratory measurements

Vitamin D concentrations were measured in all 82 patients included in the study by a chemiluminescence assay according to the manufacturer's protocol (Liaison, DiaSorin, Italy).

Comparisons

VD serum concentrations were compared between both groups (dengue patients vs control group). As a second analysis, all patients were divided in quintiles according to their VD levels and the prevalence of DWWS was compared in all 5 groups. Finally, as the third analysis of our data, infected patients and the control group were subdivided according to the VD serum concentrations based on the "cut points" suggested by the Endocrine Society: Sufficiency > 30 mg/dL; insufficiency 20 – 30 mg/dL and deficiency, < 20 mg/dL [13].

Sample processing and analysis were performed in a private mid-sized clinical laboratory (Laboratorios Galindo SC). This study was approved by the ethics committee of Oaxaca Site Management Organization.

Statistical analysis

All statistical analysis were performed using the R software version 3.5.3. As this was a non-linear study with unpaired samples, compliance with the normality of the results of the vitamin D concentrations and cut points in all analysis was verified with the Kolmogorov-Smirnov test, followed by the verification of their variances with the F and Levene tests and finally, a Student's T test for unpaired samples with different variance was used to compared the VD serum concentrations of the study groups.

Results

Demographic and clinical characteristics of patients are given in Table 1.

Clinical and demographic characteristics	Patients with DWWS (N = 41)	Control group (N = 41)
Age*	40.2 years	40.7 years
Fever	41 / 41 (100%)	0 / 41
Headache	29 / 41 (70.7%)	0 / 41
Myalgia/arthritis	22 / 41 (53.7%)	0 / 41
Retro orbital pain	5 / 41 (12.2%)	0 / 41
Rash	8 / 41 (19.5%)	0 / 41

Table 1: Demographic and clinical characteristics of patients.

*Median values.

Vitamin D concentrations

Serum 25-hydroxyvitamin D were investigated in all 82 patients included in the study (41 dengue patients and 41 control patients). Vitamin D concentrations were found to be significantly lower

($p = 0.0231$) in patients with DWWS, median value 18.09 mg/dL and SD 4.96, as compared to healthy controls, 21.14 mg/dL and SD 6.84 (Figure 1). When both groups of patients were compared based on their gender, only in females there was a statistically significant difference in the concentration of VD between the dengue patients group and that of its control group (Table 2).

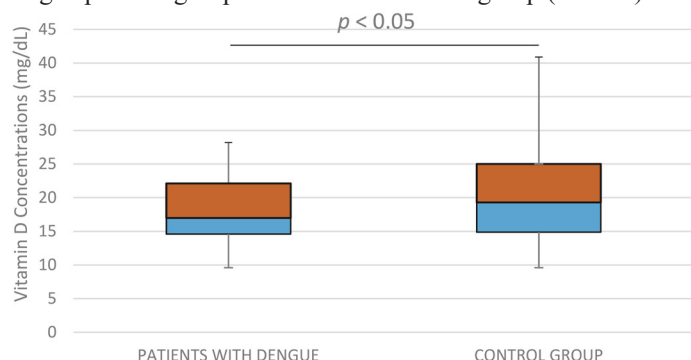


Figure 1: Serum vitamin D concentrations (mg/dL) in patients with DWWS and in the control group (healthy individuals).

Females N = 25			Males N = 16		
Patients with DWWS	Control group	p	Patients with DWWS	Control group	p
17.12 SD = 4.77	21.58 SD = 7.53	0.0220	19.6 SD = 5.04	20.5 SD = 5.77	0.5281

Table 2: Vitamin D concentrations according to gender in mg/dL.

To further determine if the prevalence of dengue cases was influenced by serum concentrations of vitamin D, all 82 patients included in the study were grouped in quintiles according to their levels of VD and the prevalence of dengue infections was determined in each group; the group with the highest levels of VD (Group 1) had the smallest prevalence of cases of all quintiles, whereas the group with the lowest concentrations (Group 5) had the highest prevalence of infections (Figure 2). When the percentage of cases in these two groups were compared to each other, a statistically significant difference between them was found ($p < 0.050$).

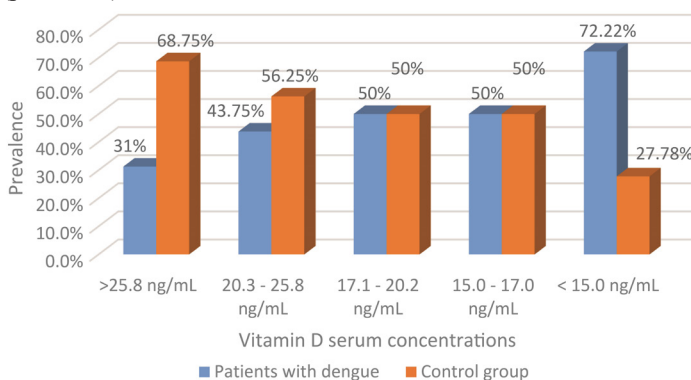


Figure 2: Prevalence of patients with DWWS against the control group among different concentrations of serum vitamin D.

As the third and final analysis of the possible relationship between DWWS infections and the serum concentrations of vitamin D,

each of the two groups of patients were classified based on the three VD “cut points” levels suggested by the Endocrine Society: sufficiency, insufficiency and deficiency. Of all patients analyzed, only 5 (6.2%) were found to have levels above the sufficient stage suggested and all five were in the control group. As can be inferred from Figure 3, the majority of patients (29/48, 60.4%) with deficient serum concentrations of VD tested positive for and had symptoms of DWWS; analysis of this particular sub-group of patients indicate that there is also a statistically significant difference, independently of gender, between vitamin D-deficient patients with DWWS and those of the control group ($p < 0.05$).

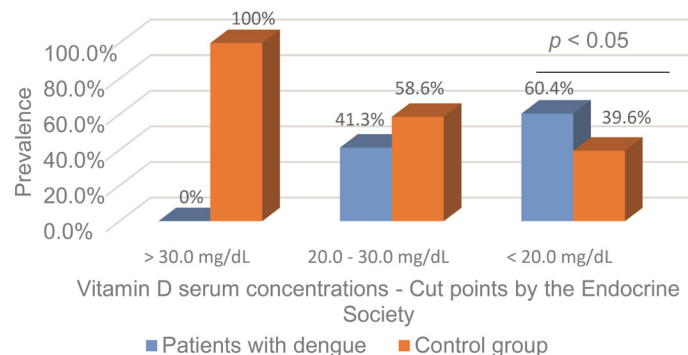


Figure 3: Prevalence of patients with DWWS against the control group among serum vitamin D concentrations suggested by the Endocrine Society [13].

Discussion

An estimated 390 million individuals worldwide are infected annually by dengue virus (1). Although many efforts have been made to elucidate the underlying mechanisms of dengue, the pathogenesis of the infection has not been fully elucidated; however, the inflammatory response seems to play a critical role in the development and outcome of the disease, thus vitamin D and its recently identified function as an immune modulator by means of its anti-inflammatory properties [14] might play a key role in controlling the progression of the disease.

The results obtained from the current study indicate that patients infected with DENV show statistically significant lower VD levels ($p < 0.050$) (Figure 1) as compared to the sex and age-matched control group, suggesting that lower concentrations of VD might be a risk factor for the development of dengue. Giraldo et al have shown that monocyte-derived macrophages exposed to high doses of vitamin D exhibited higher resistance to dengue infection [11]; thus, reduced VD concentrations might increase the risk of patients developing symptomatic dengue by means of higher cellular vulnerability to infection. Furthermore, treatment of dengue-infected patients with vitamin D indicate that the administration of high doses of VD decrease the likelihood of developing severe dengue, an additional evidence that suggests that VD may have anti-viral properties [12]. An additional discovery supporting the dengue infection – Vitamin D relationship is that during an infection by this virus one of the upregulated serum proteins is the VD receptor [15].

Different reports from Mexico and Asian countries, where dengue fever is endemic, have shown that female patients are under higher risk to develop the infection [16-19]. In our study, when serum VD concentrations of male and female patients were compared between infected individuals and control groups of the same gender, only in females a statistical difference between both groups was found (Table 2), suggesting that low concentrations of VD might play a larger role in the disease development in this group of patients. However, as VD concentrations between male and female patients shows no difference between both groups (20.02 vs 19.35 mg/dL, respectively), additional factors might contribute in the overall risk to develop dengue.

Two additional validations to our hypothesis between the relationship between serum VD concentrations and DWWS were the results obtained from our analysis of the prevalence of patients with dengue compared to a) serum VD concentrations quintiles and to b) VD concentrations suggested by the Endocrine Society. As seen in Figure 2, the prevalence of dengue cases increased from the first quintile (Highest serum VD concentrations) to the last one (Lowest concentrations) with statistical significance of $p < 0.050$ when the extreme quintiles were compared to each other. Figure 3 also shows an increase in patients with DWWS from 0% cases when patients have sufficient (> 30 mg/dL) serum levels of vitamin D to a high prevalence (60.4%) in the insufficient (< 20 mg/dL) group. Furthermore, the majority of patients with DWWS were found in the vitamin D deficient group (29/41) and in this group in particular, when the cases of patients with dengue were compared to sex and aged-matched controls, a statistical difference between both groups was found ($p < 0.050$).

The results of our study correlate with those obtained by Fatima et al in Pakistan [20] but are the opposite to those reported by Alagarasu et al in India [21], indicating that further studies are needed to understand these conflicting results. However, the possible association between the development of DWWS and low serum levels of VD is of no surprise as different reports have shown that this prohormone present antiviral activities against different viruses such as Herpes and Influenza virus and in the case of human immunodeficiency virus infections, research has shown an association between vitamin D levels with progression of the disease, survival times of HIV patients, CD4⁺ T cell counts and inflammatory responses [22]. In addition, since dengue is associated with imbalanced production of proinflammatory cytokines, vitamin D could play an important role in modulating the inflammatory responses during the infection. Furthermore, “it was shown in a small Vietnamese population where dengue is endemic that the low frequency of a dimorphic (T/t) “t” allele in the VD receptor gene (VDR) was associated with dengue disease severity, suggesting a protective role of VDR activity against dengue disease progression” [23].

Conclusion

In conclusion, all three different analysis that were performed in the study suggest a possible association between low serum levels of vitamin D and the development of dengue without warning

signs, particularly in women; however, more and larger studies are needed to confirm our findings.

Acknowledgments

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