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Original Article

Comparison of Vitamin D Levels in Patients with Dengue Haemorrhagic Fever and Dengue Fever

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Abstract

Background: To compare vitamin D levels in patients of dengue hemorrhagic fever with dengue fever

Methods: A total of 50 patients with diagnosed dengue fever, who fulfilled the inclusion criteria were enrolled in the study. Patients were divided into two groups having 25 participants each; one group had Dengue fever (DF) while the other was suffering from dengue hemorrhagic fever (DHF). Vitamin D was estimated by chemiluminescence method. Pearson's Chi square was applied to compare the proportion of patients in each study group. Relative Risk was measured along with 95% confidence interval. Significant p- value was < 0.05.

Results: Mean age of patients was 37.79 ± 15.2 years (range: 16-90 years). There were 74% males and 26% females. Mean vitamin D levels in dengue fever patients were higher (21.5 ± 13.6 ng/ml) as compared to Dengue hemorrhagic fever (12.4 ± 5.6 ng/ml). Difference was statistically significant (p=0.003).

Conclusions: Vitamin D may have a role in management of dengue fever. Low levels of vitamin D might be associated with dengue hemorrhagic fever.

Key Words: Vitamin D, Dengue fever, Dengue hemorrhagic fever

Introduction

Dengue is a viral infection in humans which spreads by mosquitoes around the world. It is one of the serious global health challenges that have emerged now a days. Dengue is a flavivirus. It has four serotypes (DV-1, DV-2, DV-3, and DV-4). People suffering from Dengue infection may be asymptomatic or having undifferentiated fevers. Other mild form of the disease is dengue fever (DF). Small proportions of patients suffer from dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) that may be fatal. Few studies have shown altered plasma concentrations of vitamin D are associated with the pathogenesis of dengue infection. DENV infection has been reported in over 100 countries and 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.[1] Dengue is a febrile illness and is responsible for causing morbidity throughout the tropical and subtropical regions of the world. There are more than 70 members of Flaviviridae, in which DENV is a single positive-stranded RNA virus transmitted by the mosquitoes Aedes aegypti and Aedes albopictus.[1,2] There are four different serotypes of DENV (DV-1, DV-2, DV-3, and DV-4) that cause dengue fever.[3]

The clinical manifestations of DENV infection may vary from asymptomatic infection, undifferentiated fever, or it can present with DF,DHF, and DSS which are the three clinical manifestations in humans. DF is the least severe, non fatal form which presents with flu like symptoms. DHF includes hemorrhagic manifestations such as spontaneous bleeding, decreased platelet count, and evidence of increased vascular permeability noted as increased hemoconcentration or pleural effusion or ascites. DHF may progress to hypovolemic shock known as DSS which is characterized by a rapid, weak pulse, narrow pulse pressure (≤20 mm Hg) or hypotension with cold, clammy skin in the early stage of shock. [4] Both DHF and DSS can be life-threatening and cause mortality in many cases. It has been found in a study that dengue virus titers are significantly higher (upto 1000 fold) in patients with DHF and DSS as compared to those having DF in the initial phase of infection.[5] Severity of Dengue virus infection is more common in children than adults.[6]

The outcome of DENV infection is determined by multiple factors including viral virulence, host genetics and host immune responses[7]. The pathophysiology of DENV in the body and the host's immune response are not completely understood. It is believed that virus

binds itself to host cells and this binding is mediated by major viral envelope (E) glycoprotein present in the virus[8]. This is followed by entering of virus in the cell and its replication. It is believed that monocytes play an important role and they produce interferon-α (IFN-α) and IFN-β.[9, 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. These infected cells are attacked by CD4+ and CD8+ T lymphocytes resulting in release of lymphotoxins, anti inflammatory cytokine (IL-10), tumor necrosis factor-α (TNF-α) and interferon-γ, all contributing to the pathogenesis of disease. [11-13] There are various immunomodulators, the presence or absence of which influence the outcome of the disease by activating T cells, antibodies and cytokines. [14]

Primary infection induces lifelong immunity in the individual to that particular serotype but not to secondary infection by a different serotype. If secondary infection occurs, disease severity may be exacerbated by CD4+ and CD8+ T lymphocytes and preexisting antibodies[15]. Various studies have shown a relationship between secondary DENV infection and its hemorrhagic manifestations.

Host nutritional status is a strong predictor of immunity.[16] 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.[17] Vitamin D has an antiviral activity and regulates inflammatory response. By binding to VDR (Vitamin D receptor), it influences gene expression by translocating to the nucleus. As a result of which phagocytic activity of macrophages is increased which induce antimicrobial peptide gene expression contributing to innate immune responses.[18] Vitamin D also enhances Th2 cytokine and IL-10 responses, whereas it inhibits cytotoxic T cell and T-helper 1 (Th1) cell responses. [19]

A study from Vietnam has shown the association of vitamin D receptor gene polymorphisms with susceptibility to DHF.[20] Thus Vitamin D could modulate pathways leading to dengue hemorrhagic fever/dengue shock syndrome.

Patients and Methods

A total of 50 patients with diagnosed dengue fever, who fulfilled the inclusion criteria were enrolled in the study from September,2016 to November,2016 from Benazir Bhutto Hospital after getting ethical review board approval. Patients were divided into two groups having 25 participants each; one group had Dengue fever (DF) while the other was suffering from dengue hemorrhagic fever (DHF). Data was recorded in self-structured questionnaire.Data was analyzed using SPSS Version 21. Numerical data like age, Vitamin D levels was presented as mean ± standard deviation. Categorical data was presented as frequencies. Pearson's Chi square was applied to compare the proportion of patients in each study group. Relative Risk was measured along with 95% confidence interval. Significant value was < 0.05.

Results

During the study period of three months, total 50 patients were included in the study after taking informed consent. Mean age of patients was 37.79 ± 15.2 years (range: 16-90 years). There were 37 (74%) males while 13 (26%) were females. Out of total 50 patients, 25 patients were diagnosed having Dengue fever and 25 having dengue hemorrhagic fever. **(**Table 1). **C**omparison of clinical features as well as Vitamin D levels in both groups was done. Mean vitamin D levels in dengue fever patients were higher (21.5 ± 13.6 ng/ml) as compared to dengue hemorrhagic fever (12.4 ± 5.6 ng/ml). Independent sample t test showed that the difference is statistically significant (p=0.003) (Table 2).

Table-I: Age and Gender of the patients (n=50)

Variable	Dengue fever(n=25)	Dengue Hemorrhagic fever(n=25)	p- value
Age(mean ± SD)	39.1± 17.6	34.04 ± 11.89	0.05*
Gender n(%)			
Male	15(60)	22(88)	0.05#
Female	10(40)	3(12)	

* Independent sample t test;# Chi square test

Table 2: Vitamin D levels of the patients (n=50)

Variable	Dengue fever(n=25)	Dengue Hemorrhagic fever(n=25)	p-value
Vitamin D Levels (ng/ml)	21.5± 13.6	12.4 ± 5.6	0.003*

* Independent sample t test;# Chi square test

Discussion

In present study, Vitamin D levels were higher in patients with DF (21.5 ± 13.6) as compared to the patients with DHF (12.4 ± 5.6) with significant P

value= 0.003. Vitamin D3 is a potentially useful antiviral compound and is known to affect the disease process of dengue by altering immune responses.[21] There is an increased susceptibility of viral infections and autoimmune diseases with vitamin D deficiency.[22] Vitamin D causes monocyte differentiation and T-cell activation by binding to vitamin D receptors and activating vitamin D-responsive genes in the body. Vitamin D inhibits cytotoxic T cell responses as well as T-helper 1 (Th1) cell. It also enhances responses of IL-10 and Th2 cytokine. [23]

In many larger studies, risk of several other infections is lowered by Vitamin D supplementation. In a study, two cases of immune thrombocytopenia were reported to be successfully treated by high-dose vitamin D supplementation and hydroxychloroquine. It was suggested that vitamin D3 down regulated CD4+ T cells and up regulated T-regulatory cells, which eventually restored platelet levels.[24] White blood cells also become activated by binding of vitamin D to VDR (vitamin D receptors) present on their surface. Hence deficiency of vitamin D can lead to leucopenia. This could be another explanation that decreased levels of vitamin D increases the clinical risk and severity of DENV infection.

A recent study conducted by Ahmed S, Finkelstein JL, et al in 2014 emphasized the importance of micronutrients including vitamins D and E in dengue virus infection [17]. The relationship between vitamin D and DENV infection in patients with dengue have been investigated in multiple studies.. In another study Guardo PH, Medina F, et al found that vitamin D3 significantly reduced the levels of pro-inflammatory cytokines (TNF-α, IL-6, IL-12p70 and IL-1β) produced by infected U937 cells and, exposure to Vitamin D3 significantly reduced the number of infected cells, particularly in monocytic cells, and lowered the production of pro-inflammatory cytokines.[25] This study showed that inhibition of DENV infection is directly related to the dosage of vitamin D3.

Another study conducted at Benazir Bhutto Hospital, Rawalpindi showed significant therapeutic role of Vitamin D in progression of DF to DHF. Of 124 patients selected, those who received Vitamin D progressed less to DHF (1.6%) as compared to those who did not receive it (27%).[26]

Alagarasu showed that vitamin D might induce its effects on Fcγ-receptor expression. Fcy receptors are responsible for entry of virus in human cells. As a result of which patients who become secondarily infected with dengue fever have possibly higher viral load and have higher chances of development of DHF and DSS. Hence Vitamin D might influence viral entry into cell. [27]

There are a few smaller studies that show relationship between DENV infection and vitamin D receptor polymorphisms. Due to polymerization, there is defect in signaling of vitamin D receptor which results in inadequate response leading to decreased Interleukin -10 levels and raised Tumor Necrotic Factor-α levels, eventually causing leading to DHF. This is in contrast to a large study conducted in Vietnam known as Genome-wide association study (GWAS) which failed to find association between vitamin D receptor genes and DENV infection.[28] However GWAS study was about DSS and was conducted in children.

It is believed that reduction in severity as well as progression from dengue fever to more severe forms such as DHF and DSS is influenced by micronutrient supplementation or host nutritional status. Because of insufficient data, there are very few studies that make any specific recommendations about Vitamin D3 levels, micronutrients including Vitamin D3 and DENV infection. Additionally, there is limited information about relationship between beneficial effect of nutritional supplements including vitamin D and it's the optimal timing during the course of the illness.

Conclusion

Vitamin D may have a role in management of dengue fever. Low concentrations of vitamin D might be associated with dengue hemorrhagic fever.

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