<u>Original Research Article</u>

A Cross Sectional Assessment to Determine the Levels of Vitamin D and Genetic Variations in Vitamin D Receptors with the Dengue Virus Infection

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Abstract

Aim: Determining the levels of vitamin D and genetic variations in vitamin D receptors with the dengue virus infection.

Methodology: The present cross-sectional study was conducted at the Department of General Medicine, Netaji Subhas Medical College and Hospital, Bihta, Patna, Bihar, for the period of 1 year. Children with Dengue who were admitted to ICU, Department of Paediatrics, Niloufer Hospital during the study period were included in this study. Study sample Size was 100. Children fulfilling the diagnostic criteria for Dengue Fever were enrolled in the study after getting informed consent from the parents/guardians. We included Age 1 month-12 years. Children fitting into the diagnostic criteria of Dengue Fever-Dengue NS1 Antigen positive or Dengue IgM Antibodies Positive. Parents/guardians of children with the criteria mentioned above who are willing to give informed consent. The following investigations were done at admission (phase 1), 48 hrs after admission (phase2), at discharge (phase3) Complete Blood count, Hematocrit, Serum Vitamin D levels at hospital admission. We followed up with all the children until they were discharged. We included all 100 children during that period and sample size was justified at alpha error 0.05, power 80. The treatment process and investigations were documented. The data were entered in Microsoft Excel 2010 version and analyzed.

Results: Among the population studied, 51% were between the age group of 6-10 years, 27% be-longed to the age group of 11-14 years,13% belonged to the age group of 2-5 years, and 6% belonged to the age group 1 month to 12 months. Among the population studied, 54% were males and 46% were females. Among the population studied, 49% had positive, Dengue IgM antibodies 27% were positive for Dengue NS1 Antigen and 24% were positive for both. Among the population studied the mean Hemoglobin values were 12.97± 2.54 gm/dl. They decreased to 11.22±1.23 gm/dl before discharge. The mean Hematocrit values were 39.2±5.83 after admission, and they decreased to 35.9±3.64 before discharge. The mean platelet values on admission were 77,402 926 cell/cumm Among the population studied Dengue Fever was present in 64%, Dengue Fever with warning signs was present in 25%,Severe Dengue was present in 11%. Among the population studied the overall mean of Serum Vitamin D levels was 17.95ng/ml. The mean of Serum Vitamin D levels was 21.23 ng/ml in cases with Dengue Fever. The mean of Serum Vitamin D level was 17.34 ng/ml in cases with Dengue Fever with warning signs. The mean of Serum Vitamin D levels was 10.8 ng/ml in cases of severe Dengue.

Conclusion: The Serum Vitamin D levels were decreasing with the severity of Dengue illness increasing and association of this is statistically significant. Decreasing levels of mean Hemoglobin were associated with increasing severity of Dengue illness and decrease was statistically significant. Decreasing levels of mean Hematocrit levels were associated with increasing severity of Dengue illness and decrease was statistically significant. Mean platelet levels were decreasing as the severity of Dengue was increasing. The decrease in the levels of means was also statistically significant. So from this study, it can conclude that with severity of dengue fever, serum vitamin D, hemoglobin, and hematocrit levels decrease.

Keywords: Hemoglobin, Hematocrit, Dengue, Vitamin D

Introduction

Dengue is an arthropod-borne viral disease caused by the Dengue virus (DENV). DENV is an RNA virus belonging to the genus Flavivirus. DENV changes genetically during natural transmission and significant biological differences have been seen between strains of the same serotype. It is a debilitating condition with high fever and break-bone pain. Infection can result in Dengue fever (DF), Dengue Hemorrhagic Fever (DHF) status, or dengue Shock Syndrome (DSS). Infection with any of the serotypes produces only partial immunity to other serotypes [1]. The World Health Organization (WHO) renders dengue as a major public health challenge globally, particularly in tropical and sub-tropical nations. Dengue has grown several folds worldwide, aided by the increased population growth rate, unplanned urbanization, global warming, frequent air travel, inefficient mosquito control, and lack of health care facilities.

The incidence of dengue has grown in the recent decade globally. An eightfold rise has been observed in the number of cases reported to WHO over the last two decades, from 505,430 cases in the year 2000, to over 2.4 million in the year 2010 and 5.2 million in the year 2019 [2] . According to National Vector Borne Disease Control Programme, in 2020 (till September 30), the total cases of dengue detected were 16439 out of which 12 died,

and in Uttar Pradesh, a total of 212 cases of were detected, of which 1 died [3] . High incidence, ranging between 21 and 50 per million, was reported for the states of Punjab, Gujarat, Karnataka, Kerala, Tamil Nadu, and Orissa [4].

Vitamin D3 plays a crucial role in the immune system, increasing the phagocytic capacity of macrophages and enhancing anti-microbial peptide gene expression, contributing to innate immunity. Vitamin D3 is a powerful immune-modulator affecting both innate and adaptive immune responses [5]. Its deficiency is mainly associated with an increased risk of cancer, influenza, tuberculosis, and human immunodeficiency virus infection. Vitamin D3 was also reported to influence the expression of DENV receptors in immune system cells.

Vitamin D has been reported to influence the expression of dengue virus entry receptor, dendritic cell specific intercellular adhesion molecule-grabbing non integrin (DC-SIGN) and FCγRIIA in immune cells [6-8]. A study reported higher levels of vitamin D in dengue virus infected patients compared to healthy controls [9]. However, the role of vitamin D serum level in dengue pathogenesis and the association of VDR gene polymorphisms with the clinical severity of dengue infection has not been extensively studied in an Indian context.

Vitamin D supplementation has been beneficial in the treatment of infections by hepatitis C virus, an RNA virus that shares some characteristics with dengue virus (DENV) [9]. In view of the above, this study was undertaken to determine the levels of vitamin D and genetic variations in vitamin D receptors with the dengue virus infection.

Materials and Methods

The present cross-sectional study was conducted at the Department of General Medicine, Netaji Subhas Medical College and Hospital, Bihta, Patna, Bihar, for the period of 1 year. Children with Dengue who were admitted to ICU, Department of Paediatrics, Niloufer Hospital during the study period were included in this study. Study sample Size was 100. Children fulfilling the diagnostic criteria for Dengue Fever were enrolled in the study after getting informed consent from the parents/guardians. We included Age 1 month – 12 years.

Children fitting into the diagnostic criteria of Dengue Fever – Dengue NS1 Antigen positive or Dengue IgM Antibodies Positive. Parents/guardians of children with the criteria mentioned above who are willing to give informed consent.

Children with immunodeficiency, malnourishment, congenital anomalies. Patients with Dengue Fever in combination with coexisting tropical infections such as malaria or typhoid were not included in the study.

Methodology

Children with Dengue Fever satisfying the inclusion criteria were enrolled in the study after getting written informed consent from the parents/guardians.

The following investigations were done at admission (phase 1), 48 hrs after admission (phase 2), at discharge (phase 3) Complete Blood count, Hematocrit, Se-rum Vitamin D levels at hospital admission. We followed up with all the children until they were discharged. Four children were going against medical advice few days before discharge. We included all 100 children during that period and sample size was justified at alpha error 0.05, power 80.[3] The treatment process and investigations were documented. The data were entered in Microsoft Excel 2010 version. Data were analyzed using Microsoft Excel 2010 and Epi Info 7.2.0. We assessed significance at a 5% level of significance.

Results:

Among the population studied, 51% were between the age group of 6-10 years, 27% be-longed to the age group of 11-14 years, 13% belonged to the age group of 2-5 years, and 6% belonged to the age group 1 month to 12 months. Among the population studied, 54% were males and 46% were females. Among the population studied, 49% had positive, Dengue IgM antibodies 27% were positive for Dengue NS1 Antigen and 24% were positive for both.

Table 1: The mean CBP parameters at different levels

Parameter	Hb	Hematocrit	Platelets
Phase I	12.97± 2.54	39.2±5.83	77402±64825.4
Phase II	11.94±2.86	37.1±5.82	69995±61184.84
Phase III	11.22±1.23	35.9±3.64	166926.73 ± 83783.36

Among the population studied the mean Hemoglobin values were 12.97± 2.54 gm/dl. They decreased to 11.22±1.23 gm/dl before discharge. The mean

Hematocrit values were 39.2±5.83 after admission, and they decreased to 35.9±3.64 before discharge. The mean platelet values on admission were

77,402 926 cell/cumm Among the population studied Dengue Fever was present in 64%, Dengue Fever with warning signs was present in 25%,Severe Dengue was present in 11%. Among the population studied the overall mean of Serum Vitamin D levels was 17.95ng/ml. The mean of Serum Vitamin D levels was 21.23 ng/ml in cases with Dengue Fever. The mean of Serum Vitamin D level was 17.34 ng/ml in cases with Dengue Fever with warning signs. The mean of Serum Vitamin D levels was 10.8 ng/ml in cases of severe Dengue.

Table 2: Serum Vitamin D levels according to the grade of Dengue Fever

Parameter	Serum Vitamin D levels
Overall	17.95 ± 5.96
Dengue Fever	21.23±5.33
Dengue Fever with warning signs	17.34±4.53
Severe Dengue	10.8±2.35

As the Serum Vitamin D levels were decreasing, the severity of Dengue illness was increasing and association of this is statistically significant with a P-Value of <0.05. Among the population studied, decreasing levels of mean Hemoglobin were associated with increasing severity of Dengue illness and decrease was statistically significant with a P-value of <0.05.Among the population studied, decreasing levels of mean Hematocrit levels were associated with increasing severity of Dengue illness and decrease was statistically significant with a P-value of <0.05. Among the population studied, the mean platelet levels were decreasing as the severity of Dengue was increasing. The decrease in the levels of means was also statistically significant, with a P-value of <0.05.

Discussion:

The available evidence on the link between vitamin D status and dengue infection is scanty and inconsistent [10-12]. Fatima et al. reported low vitamin D status among adults with dengue fever (DF) compared to unmatched healthy controls [10]. The sample included a case-to-control ratio of 1 : 1 (15 subjects in each group), and vitamin D level was analyzed using a spectrophotometric method. It is important to keep in mind that problems of accuracy of laboratory methods could lead to systematic errors and, thus, may cause variability of research findings. Alagarasu et al., in a recent study in India, reported contrasting results [11]. The study compared vitamin D levels in adults with DF ($n = 83$) and DHF ($n = 29$) with healthy individuals. The adults with DF/DHF had higher serum 25(OH)D levels than healthy controls, which was statistically significant [12].

Villamor et al. studied the association of initial vitamin D status at the febrile phase and its relationship with the progression of the disease to DHF/DSS among children and adults [12]. The study concluded that low serum 25 (OH)D levels during the febrile phase predicted decreased odds of progression to DHF/DSS. Overall, these inconsistencies among studies may be explained by differences in case definitions, dengue virus serotypes, age of the participants, and disease severity.

The majority of the population studied were aged 6 to 10 years old, which is similar to Dissanayake S et al 2021, [13] whose mean age of cases was 8.8 years while the mean age of controls was 7.9 years. In the present study, among the population studied, 54% were males and 46% were females. In Dissanayake S et al study [13], Males were 55%, females were 45% among cases. Males were 48%, Females were 52% among controls. According to Chakravarti A et al. 2020, [14] 62.8% of children were male,

while 37.2% were females. This can usually be explained by the higher mobility of male children in developing countries. Dengue serology proportions were almost similar to Chakravarti A et al 2020. [14]

In our study Hb, HCT and platelets were decreasing as the severity of the disease increased. Hb, HCT, and Platelets are statistically highly significant. Interestingly, Chakravarti A et al 2020,[14] found that Hb HCT and platelets were not significantly associated with severity of dengue illness. HB and platelets were associated with a 2018, [15] p-value of 0.02 and 0.03 respectively according to Javed R et al. Among the population studied, Dengue Fever was present in 64%. Dengue Fever with warning signs was present in 25%, Severe Dengue was observed in 11% which is quite Similar to Fatima H et al 2018. [10] In the present study, among the population studied, the overall mean of Serum Vitamin D level was 17.95 ng/ml. The mean of Serum Vitamin D level was 21.23 ng/ml, in cases with Dengue Fever.Patients with Dengue Fever and warning signs had a mean serum Vitamin D level of 17.34 ng/ml.The mean of Serum Vitamin D level was 10.8 ng/ml, in cases with severe Dengue Fever. The difference between the means of Serum Vitamin D levels according to the grading of Dengue Fever was statistically significant. As serum Vitamin D levels are decreasing, the severity of Dengue Fever is increasing, and the association is statistically significant. [16]

Dissanayake S et al 2021, [13] 3reported in a multivariate logistic regression, the likelihood of Having Vitamin D [25(OH)D < 20 ng/mL] was 3.6 times higher in cases compared to controls (Odds Ratio (OR): 3.65, 95% confidence interval (CI): 1.461, 9.102, p < 0.006). When serum 25(O.H.)D was used as a continuous independent variable, the strength of the association between DHF/DSS and serum 25(O.H.)D was weak but statistically significant; the likelihood of having DHF/DSS is 0.94 times less with 1 ng/mL increase in serum 25(O.H.)D (OR: 0.940, 95% CI: 0.887, 0.995, p < 0.03). Javed R et al 2018,[15] Serum Vitamin D levels were higher among those with a greater severity of disease with a p-value of 0.031. Villamor E et al 2017, [10] noticed low serum 25(OH)D concentrations in DF patients predict decreased odds of progression to DHF/DSS.

Conclusion:

The Serum Vitamin D levels were decreasing with the severity of Dengue illness increasing and association of this is statistically significant. Decreasing levels of mean Hemoglobin were associated with increasing severity of Dengue illness and decrease was statistically significant. Decreasing levels of mean Hematocrit levels were associated with increasing severity of Dengue illness and decrease was statistically significant. Mean platelet levels were decreasing as the severity of Dengue was increasing. The decrease in the levels of means was also statistically significant. So from this study, it can concluded that with severity of dengue fever, serum vitamin D, hemoglobin, and hematocrit levels decrease.

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