

## ASSOCIATION BETWEEN SERUM ASCORBIC ACID LEVELS AND SEVERITY OF DENGUE IN CHILDREN-A CASE CONTROL STUDY

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### Abstract

**Background:** Dengue fever is a viral infection caused by arthropod-borne virus of the Flaviviridae family. The disease is spread by the bite of Aedes mosquitoes. The WHO has considered dengue as a global threat in tropic and subtropical nations. It is important to identify a modifiable risk factor for development of severe dengue to prevent occurrence of severe manifestations. **Objective:** To determine the correlation of serum ascorbic acid level with severity of dengue fever in children **Materials and Methods:** The case control study was done in the Department of Paediatrics, PSGIMSR for a period of one year from February 2018 to January 2019. All the children and adolescents between 1-15 years with confirmed dengue fever were taken as cases. Children of similar age group who were admitted for other reasons were taken as controls. The final sample size was 109, where cases were 69 and controls were 40. Baseline characteristics like name, age, sex, location and laboratory parameters were taken. The data was collected and analysed through SPSS software. **Result:** The comparison of mean Vitamin C levels between control group and dengue children showed a statistically significant lower value in the control group when compared to the study group ( $P = 0.01$ ). The mean value of vitamin C levels (in mg/dl) in the cases [ $2.36 \pm 1.47$ ] was high when compared to the control group [ $1.73 \pm 0.99$ ] which was statistically significant. This meant that children with dengue fever had higher levels of vitamin C when compared to controls. **Conclusion:** The study concluded that higher level of serum vitamin C was seen in the dengue children compared to controls and the difference was found to be significantly significant.

## INTRODUCTION

Dengue fever is a viral infection caused by arthropod-borne virus of the Flaviviridae family, occurring in countries with temperate climate.<sup>[1]</sup> The disease is spread by the bite of Aedes mosquitoes. There are four identified serotypes of dengue virus [DENV1, DENV2, DENV3, and DENV4]. Each serotype has several genotypes – 3 in DENV1, 2 in DENV2, 4 in DENV3 and 4 in DENV4. The virus has three structural protein genes (coding for core, membrane associated and an envelope protein of the nucleocapsid) and seven non-structural proteins- NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5, of

which only NS1 antigen is found to interact with host immune system and the function of others is not well-characterised.<sup>[2]</sup>

These mosquitoes are usually day-time biters and breed in clean water. Trans-ovarian transmission of the virus [i.e., the ability of mosquitoes born out of infected mosquitoes to act as vectors] also contribute to the exponential infection occurring during outbreaks.<sup>[3]</sup> The mosquitoes usually fly only over a short distance and this is the reason why there is clustering of cases noted over a small well-defined geographical area during outbreaks.

The immune reaction to the virus is implicated in the severity of the disease. The central hallmark of

severe dengue is “capillary leaks” wherein there is increased capillary permeability causing leakage of plasma from the intravascular compartment to the interstitial and extra vascular compartment leading to the manifestations of “Dengue-shock syndrome”. The illness begins abruptly after the incubation period which usually lasts for 4 to 10 days. There are three phases during the illness viz., febrile phase, critical phase and recovery.

Vitamin C is a water soluble vitamin found in abundance in citric fruits like oranges, lemon, Indian gooseberries, tomatoes<sup>[4]</sup> Non-synthetic functions include role in immune function and absorption of nonheme iron.<sup>[5]</sup> The antioxidant function prevents free radical injury to endothelium and in addition to it collagen biosynthesis helps in wound-healing and thus helps in maintaining endothelial integrity by many mechanisms. There is a possibility that it could be low because of poor oral intake and vomiting in children with the disease. Low ascorbic acid levels in scorbutic patients have been found to contribute to plasma leaks.<sup>[4]</sup> Hence the capillary leaks in dengue maybe attributed to low levels of ascorbic acid in children with severe disease. So we hypothesized that the ascorbic acid levels in children with severe dengue could be low when compared with healthy children and children with milder forms of the disease.

#### **Aim of the Study**

The aim of the study is to assess the ascorbic acid level in children with dengue fever and to determine the correlation of serum ascorbic acid level with severity of dengue fever in children.

## **MATERIALS AND METHODS**

This case control study was conducted in the Department of Paediatrics, PSGIMSR. The study was done for a period of one year from February 2018 to January 2019.

#### **Inclusion Criteria**

All the children and adolescents between 1-15 years of age with confirmed Dengue were taken as Cases. Children and adolescents between 1-15 years of age for other causes were taken as controls.

Sample size: All the dengue confirmed children during the study period were recruited. The total cases recruited were 109 children. 40 children will be recruited from each category namely mild dengue, moderate dengue and severe dengue through convenient sampling. 40 children between the age group of 1-15 years with normal nutritional status [based on age and sex appropriate IAP growth charts and WHO criteria] without chronic illnesses will be taken as a control group.

#### **Data Collection Method**

After obtaining informed consent from a parent/guardian and assent [as applicable] from children, 2ml of blood sample was taken for measurement of ascorbic acid in all children admitted in the department of paediatrics, PSGIMSR with the diagnosis of mild dengue/moderate dengue/ severe dengue [as per NVBDCP guidelines on management of dengue] on Day 4 of illness or day of admission, whichever is earlier.

All children were monitored for the evidence of onset, progression or regression of plasma leakage in the form of pedal oedema, facial puffiness, ascites, pleural effusion and shock. All the parameters were entered in a predesigned proforma. Children and adolescents of age ranging 1-15 years who were admitted for other reasons and without chronic illnesses were taken as control. 2ml blood sample, from all study participants, was obtained in a vacutainer with heparin, plasma separated and acidified with 2ml of freshly prepared 10% metaphosphoric acid and stored at -70 °C. The samples were sent in batches transported with dry ice for analysis in Stanes laboratory, Coimbatore.

#### **Statistical Analysis**

The collected data was entered in MS excel and statistical analysis was done in SPSS 23. Continuous data was expressed in terms of Mean and Standard Deviation. Categorical variable was expressed in terms of number (Percentages). P value of <0.05 is considered as significant.

## **RESULTS**

A total of 73 dengue patients were admitted to the hospital during the study period. Of the 73 children, one child had congenital heart disease and was excluded from the study. Of the remaining 72 children, 3 parents did not give consent and hence were not included. The rest 69 children were enrolled in the study. A total of 40 controls were recruited.

Of the 69 dengue children included in the study, there were 10 [14.5%] severe cases, 31 [44.9%] moderate and 28 [40.6%] of mild dengue cases. Also, in all dengue children, serology was done before discharge and based on IgG and IgM titres, they were classified as primary dengue [only IgM positive] or secondary dengue [both IgG and IgM positive]. There were totally 26 [37.7%] primary dengue and 43 [62.3%] secondary dengue children.

**Table 1: Age wise distribution of cases and controls**

Age	Cases		Controls	
	Frequency	Percentage (%)	Frequency	Percentage (%)
0-5	24	34.8%	22	55
5-10	24	34.8%	08	20
11-15	21	30.4	10	25
Total	69	100	40	100

The mean ( $\pm$ SD) age of the dengue cases was found to be 7.29[ $\pm$ 4.34], whereas that in the control group was 6.1[ $\pm$ 4.4] in years which was not statistically significant. The least age in the cases group was 8 months and the highest age was 15 years. There were three infants included in the study group. In the control group the lowest and the highest age among the children included were 1 year and 17 years respectively. No infant was included in the control group. [Table 1] shows that the number of children with dengue in the age group of 0-5 years was less than that in the control group probably because infants and toddlers with minor illness are usually hospitalised than older children due to parental concern. Both the groups [cases and controls] had male predominance. But there was no statistical difference in the distribution of boys and girls among cases and the controls chosen.

**Table 2: Comparison of lab parameters between cases & controls**

Lab parameters	Cases	Controls
Hemoglobin (g/dl)	13 $\pm$ 1.8	12.1 $\pm$ 1.6
Hematocrit (%)	39.8 $\pm$ 5.4	37.1 $\pm$ 4.5
Platelets (Cells/cu mm)	91900 $\pm$ 81300	230000 $\pm$ 146200
SGOT(U/L)	301.76 $\pm$ 850.4	NA
SGPT (U/L)	118.07 $\pm$ 311.3	NA
Vit C levels(mg/dl)	2.36 $\pm$ 1.47	1.73 $\pm$ 0.99

The baseline parameters at admission between dengue children and the control group showed a significant difference in platelet value as expected.

**Table 3: Comparison of mean Vit C levels in dengue and in controls**

	N	Mean	SD
Dengue	69	2.36	1.47
Control	40	1.73	0.99

The comparison of mean Vitamin C levels between control group and dengue children showed a statistically significant lower value in the control group when compared to the study group (P = 0.01).

**Table 4: Comparison of baseline characteristics among mild, moderate & severe dengue**

Baseline characteristics	Control(n=40)	Mild (n=28)	Moderate(n=31)	Severe(n=10)
Age in years Mean $\pm$ SD	7.3 $\pm$ 4.51	6.99 $\pm$ 4.37	7.35 $\pm$ 4.37	7.98 $\pm$ 4.55
Male (%)	28(70%)	20(71.5)	18(58)	5(50)
Location				
Coimbatore	8	6	9	2
Tirupur	29	21	19	6
Others	3	1	3	2

The mean age was lower in the mild dengue group and higher in severe dengue. Percentage of males was significantly higher in mild dengue group. Tirupur is a neighbouring district with high dengue load and consequently, more number of cases in all 3 groups were from Tirupur district. We had cases from Nilgiris, Erode, Salem, Dindugal and also from Nagapattinam District. The mean duration of illness at admission was slightly lower in severe dengue.

**Table 5: Association between severe dengue and ascorbic acid levels**

Outcome	Vit C levels		Total	P value
	0.6-2	>2.1		
Mild Dengue	18	9	28	0.78
Moderate Dengue	22	09	31	
Severe Dengue	04	06	10	
Total				

The comparison of vitamin C levels in mild, moderate and severe dengue groups showed no statistical difference although the value is numerically greater in the severe dengue group. The normal range of vitamin C is 0.6 to 2 mg/dl according to previous studies. The normal value of Serum Vitamin C was 0.6-2 mg/dl. In our study, out of 109 children enrolled [Cases and controls included], only 7 had values less than 0.6 [4 from control

group and 3 from cases] of which only 2 children had the normal value of Serum Vitamin C was 0.6-2 mg/dl. In our study, out of 109 children enrolled [Cases and controls included], only 7 had values less than 0.6 [4 from control group and 3 from cases] of which only 2 children had  $<0.3$ . The mean value of vitamin C levels (in mg/dl) in the cases [ $2.36 \pm 1.47$ ] was high when compared to the control group [ $1.73 \pm 0.99$ ] which was statistically significant.

The clinical parameters show a significantly high PCV in the severe dengue group and elevated liver enzymes when compared to the other two groups. The outcome of treatment showed all cases improved and discharged except two in severe dengue group. One died and another was discharged against medical advice. In the severe dengue group, 4 children required colloids, 4 required inotrope support and 2 children needed mechanical ventilation.

## DISCUSSION

Dengue causes significant morbidity as well as mortality in children in India.<sup>[6]</sup> The disease has a dynamic course and the severity may vary during the course of illness. The only identified risk factor till date that could predict the progression of severity is the presence of infection-enhancing antibodies.<sup>[7]</sup> Severe dengue usually presents with hypotension that would necessitate fluid boluses, colloids, inotropes and mechanical ventilation.<sup>[8]</sup>

ECMO [Extra-Corporeal Membrane Oxygenation] and CRRT [Continuous Renal Replacement Therapy] have changed the outcomes of severe dengue that end with ARDS [Acute Respiratory Distress Syndrome] or AKI [Acute Kidney Injury]. But it requires expertise and centres with facilities.<sup>[9]</sup> Moreover, the cost of treatment is too high and burdening on the caregivers. An effective and a cheap agent that could prevent the capillary leaks associated with dengue fever and thus alter the hemodynamic imbalance that occurs in the critical phase is the need of the hour.

We considered vitamin C could be such an agent based on evidences shown in burns and sepsis models.<sup>[10-13]</sup> Hence our study aimed at finding a correlation between vitamin C levels and severity of dengue. The normal value of Serum Vitamin C was 0.6-2 mg/dl. In our study, out of 109 children enrolled [Cases and controls included], only 7 had values less than 0.6 [4 from control group and 3 from cases] of which only 2 children had had  $<0.3$ .

The mean value of vitamin C levels (in mg/dl) in the cases [ $2.36 \pm 1.47$ ] was high when compared to the control group [ $1.73 \pm 0.99$ ] which was statistically significant. This meant that children with dengue fever had higher levels of vitamin C when compared to controls, thus contradicting the proposed hypothesis of the study.

The probable reasons for the findings could be:

1. The consumption of extra fluids by dengue children in the form of fruit juices when compared to children with other viral fevers (especially respiratory infections where there is a misconception that consumption of fruit juices could worsen the infection)<sup>[10]</sup>

2. Moreover, the use of Nilavembu extract and papaya leaf extract is common in Tamil Nadu and has been advocated by the state government during dengue epidemics. The presence of alkaloids and

vitamins in these herbal preparations could have caused the increased vitamin C levels in dengue.

3. Multivitamins consumed during illness could have contributed to the significantly higher ascorbic acid levels in dengue children.

4. In previous studies in critically ill patients, it has been shown lower levels of vitamin C are associated with severity of illnesses.<sup>[11,12]</sup> Vitamin C is a powerful anti-oxidant and helps in maintaining capillary integrity. Although due to increased oxidative stress, vitamin C requirements are greater in this population, levels may be restored to normal and sometimes supra-normal, with parenteral supplementation.<sup>[14]</sup> The high levels of vitamin C in dengue children in our study can be attributed to the compensatory mechanisms to counteract free oxygen radical injury and the occurrence of capillary leaks. With good enteral supplementation of multivitamins, there could have been an acute increase in levels due to the need for increased anti-oxidant activity.

The high levels of vitamin C in dengue children in our study can be attributed to the compensatory mechanisms to counteract free oxygen radical injury and the occurrence of capillary leaks. With good enteral supplementation of multivitamins, there could have been an acute increase in levels due to the need for increased anti-oxidant activity. Though we had collected data regarding nutritional status of participants, we had not collected data regarding consumption of commercially available multivitamin supplements, fruit juices, diet and herbal preparations like papaya leaf extract or Nilavembu extract.

The levels (mean  $\pm$  SD in mg/dl) were  $2.35 \pm 1.29$ ,  $2.17 \pm 1.55$ ,  $2.99 \pm 1.62$  respectively. On comparing the mean values with one-way ANOVA, there was no statistical significance although the mean value in severe dengue was comparatively higher when compared to the other two groups. The difference in the mean between the groups can be easily made out by comparing the charts 10-12. The chart shows an increased number of children in the group of vitamin c  $> 2.1$ mg/dl in the severe dengue group when compared to the other two groups. The difference might imply the possibility of lower vitamin C levels being associated with less severe dengue again contradictory to the original hypotheses in this study. The reason for increased vitamin C levels in severe dengue children may again be explained by the need for high anti-oxidant

levels. Moreover the increased capillary permeability demands the need for increased ascorbic acid levels.

### Strengths and Limitations

1. This is the first study to estimate vitamin C levels in dengue and compare and correlate the levels in mild, moderate and severe dengue. The laboratory technician was blinded for Vitamin C analysis. Samples were labelled and sent so that it would not be possible to distinguish if it was from controls or mild, moderate or severe dengue. The estimation method was a standard protocol for estimation of vitamin C.
2. We had adequate resources and logistics required for storage and transportation of samples in our institution. Our study was institution funded. Ethical considerations were strictly adhered.
3. Our study had a few limitations. The diet, multivitamin supplements and herbal preparation intake was not documented which could have impacted on the vitamin C levels. The controls were from in-patients with minor illnesses which could have affected vitamin C levels. Vitamin C sample collection at fasting was not always possible especially in severe dengue children requiring ICU admission. HPLC method for estimation of vitamin C levels would have been more accurate, but was not economically feasible.

### CONCLUSION

With the available data in the study, it has been shown that there is a significantly higher level of serum vitamin C in dengue children when compared with controls. And also, severe dengue children have higher levels when compared to mild dengue cases. These findings are contradictory to the original hypothesis and may be explained by multivitamin and herbal preparation supplementation in these children. The study suggests that though vitamin C levels can be increased by enteral supplementation, it does not alter the course or severity of the illness.

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### Data Analysis

### Declarations

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**Ethical Approval:** Obtained (IHEC).

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