Asian Pacific Journal of Tropical Disease

journal homepage: http://www.apjtcm.com

Original article https://doi.org/10.12980/apjtd.7.2017D7-176

Effects of vitamin E supplementation on the clinical outcome of dengue fever and dengue haemorrhagic fever in children

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ARTICLE INFO

Article history:
Received 24 Jul 2017
Received in revised form 28 Aug 2017
Accepted 11 Sep 2017
Available online 24 Oct 2017

Keywords:
Vitamin E
Dengue fever
Dengue haemorrhagic fever
Sri Lankan children

ABSTRACT

Objective: To evaluate the effects of vitamin E supplementation on the clinical course of dengue fever (DF) and dengue haemorrhagic fever (DHF) in 5–12 years old Sri Lankan children.

Methods: A triple blinded controlled interventional trial was conducted at a tertiary care hospital in Sri Lanka. Five to twelve-year-old children with clinically suspected dengue infection within 84 h of onset of fever were randomly allocated to receive an age-adjusted dose of vitamin E or placebo. Standard ward management for DF/DHF was provided for both groups. Clinical, biochemical [aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum albumin, serum cholesterol, serum calcium] and haematological [white blood count (WBC), platelets, packed cell volume (PCV)] parameters were monitored regularly throughout the course of the illness.

Results: Data of 127 subjects (n = 61 in treatment group) were analysed after they were confirmed of dengue infection. Treatment and placebo groups both were similar. The temporal distribution patterns of WBC, platelets, serum albumin and serum cholesterol were higher, while PCV, serum AST and ALT levels were lower in the treatment group compared to placebo group. PCV on Day 3.5, WBC on Day 6, ALT on Days 4 and 5, AST on Days 3–5 and albumin on day 5 were significantly improved in the treatment group. There was no difference in the duration of hospital stay or occurrence of leaking. However, the duration of leaking was significantly lower in the treatment group.

Conclusions: Treatment with vitamin E shows a significant improvement in clinical, haematological and biochemical parameters in children with DF and DHF.

1. Introduction

Dengue is one of the most important mosquito borne viral diseases, affecting approximately 50% of the world population[1]. Its incidence has increased dramatically over the last decade, and currently it is estimated to be 390 million dengue infections per year[1]. The virus has four serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) and is transmitted by the mosquito species *Aedes aegypti* and *Aedes albopictus*. It causes diverse clinical manifestations which could range from asymptomatic infection to dengue haemorrhagic fever (DHF) with shock and can be complicated by bleeding, liver failure, encephalopathy and myocarditis[2]. Currently dengue is hyperendemic in Sri Lanka[3].

The pathogenesis of dengue infection is a complex interaction between the dengue virus, host genetic and host immune factors. Initial infection with a particular serotype (primary infection) is usually asymptomatic or results in mild disease. Subsequent infection with a different serotype (secondary infection) leads to a more severe disease or DHF due to the presence of cross-reactive non-neutralizing antibodies which enhance the infection

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Ethical clearance was granted by the Ethics Review Committee of Faculty of Medicine, University of Colombo. The study was approved by the National Medicines Regulatory Authority of the Ministry of Health, Sri Lanka. The protocol was registered in Sri Lanka Clinical Trials Registry (SLCTR/2015/012). Informed written consent was obtained from parents of study participants.

The journal implements double-blind peer review practiced by specially invited international editorial board members.

of host cells expressing Fc receptors, thus exaggerating the immune response[4]. Cytokines released in this process cause endothelial dysfunction leading to increased vascular permeability, thus causing vascular 'leak', the hallmark of DHF, resulting in pleural effusion, ascites and shock[5].

There is growing evidence that oxidative stress plays a key role in dengue infection[6] similar to other viral infections such as HIV[7], viral hepatitis[7], and influenza[8].

When infected with a virus, immune system gets activated and reactive oxygen species (ROS) are generated in the neutrophils, monocytes and endothelial cells by the enzymes NADPH oxidase and myeloperoxidase using oxygen and hydrogen peroxide. These ROS are removed by antioxidants comprised of vitamins (A, C, E), glutathione and enzymes (superoxide dismutase and glutathione peroxidase). Oxidative stress occurs when the amount of oxidants (ROS) exceeds the concentration of antioxidants. Oxidants induce release of nitrous oxide, interferon alpha, tumor necrosis factor alpha and interleukin 6 which can cause apoptosis, necrosis and inflammation[9]. Oxidants can damage lipids, proteins, carbohydrates and nucleic acids resulting in cellular injury. These processes can ultimately result in organ damage and increase the risk of secondary infections[10].

Gil *et al.* demonstrated increased lipid peroxidation, reduced antioxidant activity of glutathione peroxidase and increased activity of superoxide dismutase, suggestive of increased oxidant activity and reduced antioxidant capacity in adult dengue patients[9]. Increased oxidative stress has been demonstrated in children with DHF as well[11].

Maximum oxidative stress was seen in dengue shock syndrome followed by DHF and was minimal in dengue fever (DF)[12]. Additionally the thrombocytopenia correlated with the extent of lipid peroxidation[12]. Oxidative stress is considered to play a role in liver and tissue injury in dengue infection[6,13]. Therefore antioxidants could have a place in the management[6].

Vitamin E (alpha tocopherol) is a peroxyl radical scavenger. It prevents the propagation of free radicals in tissues by reacting with them to form a tocopheryl radical. It will then be reduced by a hydrogen donor and returns to its reduced state[14]. Vitamin E, being fat-soluble, gets incorporated into cell membranes, thus protecting them from oxidative damage. In addition to being an antioxidant, vitamin E is also involved in immune function, enzyme activation and gene expression[15]. It has been clinically useful in critically ill patients[10] and non-alcoholic steatohepatitis where oxidative stress plays a role in pathogenesis[16]. Vitamin E supplementation has also shown a reduction in viral load in HIV patients[17].

Despite the high prevalence of dengue infection, there was only one controlled trial available on vitamin E supplementation in DF. Vaish *et al.* discovered a significant increase in platelet count in a small population of adult dengue patients supplemented with vitamin E[18]. However other aspects of the illness were not evaluated.

The objective of this study was to assess the effects of vitamin E on the clinical course, haematological and biochemical parameters and the development of complications in DF/DHF in children.

2. Materials and methods

Prospective randomized triple blinded control trial was carried out in the Professorial Paediatric Unit of Lady Ridgeway Hospital, Colombo, Sri Lanka from August 2015 to July 2016. Ethical clearance was granted by the Ethics Review Committee of Faculty of Medicine, University of Colombo. The study was approved by the National Medicines Regulatory Authority of the Ministry of Health, Sri Lanka. The protocol was registered in Sri Lanka Clinical Trials Registry (SLCTR/2015/012).

Children between the ages of 5–12 years, and who fulfilled the clinical criteria of DF (fever and 2 of the other clinical symptoms: headache, retro-orbital pain, myalgia, arthralgia, bone pain and haemorrhagic manifestations) were recruited, if the admission was within the first 84 hours of the onset of fever. Any child with an underlying chronic illness such as cardiac, renal or hepatic disease or on long term medications was excluded. Informed written consent was obtained from parents of study participants.

Patients were randomized into treatment and control groups according to their age category using a computer generated random number table. The age categories were 5–9 years and 10–12 years. In the treatment group, children between 5 and 9 years of age received 200 mg of vitamin E (Evion, Merk) daily and children between 10 and 12 years of age received 400 mg of vitamin E (Evion, Merk) daily from the day of admission up to 7th day of illness[19]. The control group received a commercially prepared placebo for the same period.

On admission a thorough physical examination including Hess test was performed and base line blood samples were sent for full blood count, alanine aminotransferase (ALT), aspartate transaminase (AST), albumin, calcium and cholesterol. During the hospital stay, pulse rate, pulse volume, blood pressure, respiratory rate, urine output, and temperature were monitored every 4 hours and thereafter hourly when a patient enters the critical (leaking) phase. Capillary packed cell volume (PCV) was monitored every 8–12 hours. Full blood count was repeated twice a day and ALT, AST, albumin, calcium and cholesterol were repeated once a day. Full blood count was analysed by Mindray Analyser and biochemical parameters were analysed by Kone-30 lab prime automated analyser using Biolabo reagents. All patients underwent inward ultrasound scans once the platelet count dropped below 100×10^9/L to detect leaking of plasma into chest and abdomen. All patients were clinically managed according to the national guidelines for DF/ DHF[20]. All patients were given calcium lactate supplementation (1 mmol/kg/day) as it was part of the standard treatment protocol of the unit.

Onset of leaking was determined when any of the following was

seen; rise of capillary PCV more than 20% from base line, drop in the platelet count < 100 × 10^9/L, pleural effusion or ascites in ultrasound scan and fall in serum albumin by 0.5 g/dL or serum cholesterol by 20 mg/dL. End of leaking was determined by the stabilization of clinical parameters, reduction in fluid requirement with normalizing of PCV, beginning of rise of platelet count, establishment of diuresis and development of recovery rash.

On Day 6 of the illness, serum specimens were tested for dengue IgM/IgG antibodies using SD Bioline Dengue IgG/IgM immunochromatography (Standard Diagnostics Inc, Korea – Lot no: 11AD14001). All haematological and biochemical parameters were repeated on Day 14 of the illness. If IgM/IgG were negative on Day 6 or NS1 was negative, serology assessment was also repeated at this point. All demographic, clinical and laboratory data were recorded using an interviewer administered data compilation sheet. Statistical Package for Social Sciences (SPSS) 17 was used for data analysis. Continuous outcomes that were normally distributed, were compared between the two groups using independent samples *t* test. Continuous variables that had a non-normal distribution were log transformed and independent *t* test was applied. Categorical outcomes were compared using *Chi*-square test.

3. Results

The final sample size was 127 (61 in vitamin E treatment group, 66 in placebo control group). There was no significant difference in the mean age, duration of fever, systolic or diastolic BP or the percentage with a positive tourniquet test at time of admission in the two groups (Table 1).

Table 1
Baseline characteristics of the treatment and control groups.

Characteristics on admission	Treatment[#] (*n* = 61)	Control[#] (*n* = 66)	Sig.[*]
Age (years)	8.39 ± 2.16	8.35 ± 2.03	NS
Duration of fever on admission (days)	2.82 ± 0.61	2.95 ± 0.66	NS
Tourniquet test positive	26 (42.6%)	29 (43.9%)	NS
Systolic BP (mmHg)	101.10 ± 8.01	98.90 ± 15.30	NS
Diastolic BP (mmHg)	67.20 ± 7.96	67.40 ± 7.98	NS

[#]: For continuous variables mean ± SD are given. For dichotomous variables *n* (%) are shown. [*]: Independent samples *t* test was used for continuous variables and *Chi*-square test used for categorical variables. NS: Not significant.

3.1. Clinical outcomes

Out of the total samples, about two thirds needed high dependency unit (HDU) care and about one fifth developed leaking while the occurrence of bleeding manifestations was about 7%. There was no significant difference in these outcomes in the two groups. Mean duration of HDU stay was about 2 days in both treatment and control groups. The duration of leaking was 33.50 h in the vitamin E group and 44.80 in the placebo group and this difference was statistically significant (P = 0.023) (Table 2). No significant side effects were discovered in the treatment or placebo group. All patients recovered fully from the illness and none required intensive care management.

Table 2
Clinical outcomes in the treatment and control groups.

Clinical Characteristic	Treatment[a] (*n* = 61)	Control[a] (*n* = 66)	Sig.[*]
Needing HDU care	42 (68.9%)	44 (66.7%)	0.626
Duration of HDU stay (days)	2.01 ± 1.08	1.89 ± 0.85	0.583
Occurrence of leaking	11 (18.0%)	13 (19.7%)	0.811
Duration of leaking (h)	33.50 ± 15.70	44.80 ± 5.40	0.023[#]
Occurrence of bleeding manifestations	4 (6.6%)	5 (7.6%)	0.583

[a]: For continuous variables mean ± SD are given. For dichotomous variables *n* (%) are shown. [*]: Independent samples *t* test was used for continuous variables and *Chi*-square test used for categorical variables. [#]: $P < 0.05$. HDU: High dependency unit.

3.2. Haematological outcomes

Mean white blood count (WBC) and platelet count of the vitamin E group remained higher than the placebo group from Day 3 to Day 7 of the illness (Figure 1). Day 6 WBC count was significantly higher in the vitamin E group than the placebo group. Mean PCV of the treatment group was lower than the placebo group during the course of the illness and the difference in the mean PCV between the two groups at Day 3.5 was statistically significant (Figure 1 and Table 3).

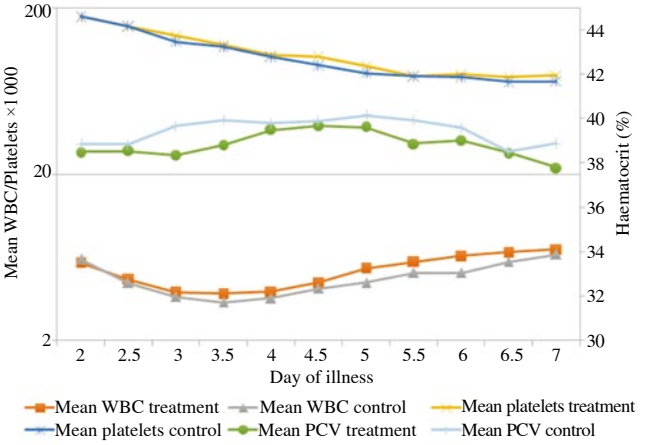

Figure 1. Variation in mean WBC, platelets and haematocrit with the day of the illness in treatment and control groups.

Table 3
Comparison of mean haematologocal outcomes in the treatment and control groups.

Day	Mean WBC		Sig.[*]	Mean platelets		Sig.[*]	Mean PCV		Sig.[*]
	Treatment	Control		Treatment	Control		Treatment	Control	
2	5.878	6.175	0.983	176.60	176.71	0.998	38.50	38.86	0.769
2.5	4.676	4.437	0.545	154.12	154.68	0.973	38.52	38.83	0.650
3	3.922	3.672	0.981	135.40	124.54	0.345	38.36	39.66	0.012[#]
3.5	3.841	3.402	0.931	119.28	116.36	0.716	38.80	39.93	0.004[#]
4	3.949	3.611	0.686	103.84	101.23	0.767	39.47	39.80	0.482
4.5	4.476	4.106	0.516	101.32	90.17	0.284	39.66	39.89	0.640
5	5.440	4.461	0.139	89.04	80.92	0.350	39.60	40.14	0.317
5.5	5.941	5.107	0.218	77.98	77.31	0.939	38.88	39.93	0.126
6	6.453	5.100	0.016[#]	79.49	76.10	0.731	39.02	39.58	0.296
6.5	6.788	5.971	0.258	76.56	71.62	0.592	38.46	38.53	0.927
7	7.073	6.596	0.639	78.50	72.22	0.559	37.81	38.87	0.219

[*]: Using independent samples *t* test. [#]: $P < 0.05$.

3.3. Biochemical outcomes

Mean AST and ALT levels in the vitamin E group remained lower than the placebo group from Day 3 to Day 7 of the illness (Figure 2). The mean ALT values of Day 4 and Day 5 were significantly

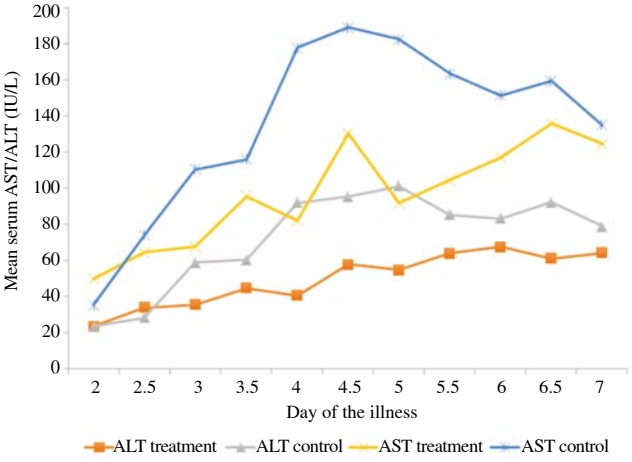

Figure 2. Variation in serum AST and ALT with the day of the illness in treatment and control groups.

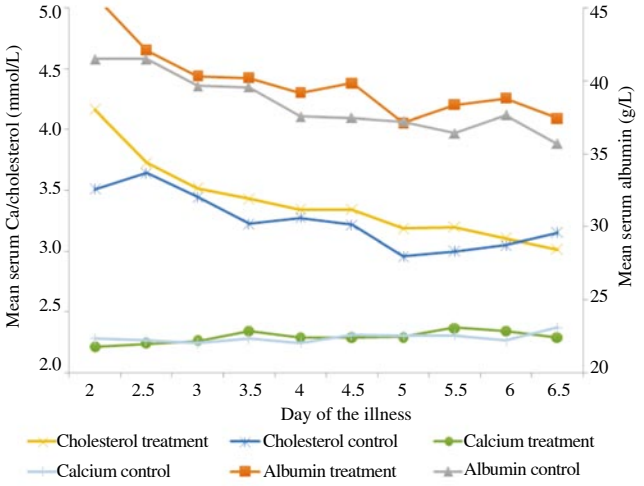

Figure 3. Variation in serum albumin, cholesterol and calcium with the day of the illness in treatment and control groups.

Table 4

Comparison of mean metabolic outcomes in the treatment and control groups.

Day	ALT (IU/L)			AST (IU/L)			Albumin (g/L)			Cholesterol (mmol/L)			Calcium (mmol/L)		
	T	C	S*	T	C	S*	T	C	S*	T	C	S*	T	C	S*
2	23.7	23.6	0.960	50.0	35.4	0.410	-	-	-	-	-	-	2.21	-	-
2.5	34.1	28.6	0.990	64.5	74.1	0.570	45.8	41.5	0.003#	4.16	3.51	0.08	2.21	2.28	0.38
3	35.8	58.9	0.150	67.7	110.5	0.020#	42.1	41.5	0.490	3.72	3.64	0.72	2.24	2.26	0.52
3.5	44.9	60.6	0.410	95.6	116.0	0.330	40.3	39.7	0.420	3.52	3.44	0.67	2.26	2.24	0.60
4	40.8	92.0	0.008#	82.1	117.7	0.005#	40.2	39.6	0.350	3.43	3.22	0.29	2.34	2.28	0.15
4.5	57.9	95.4	0.500	130.5	189.1	0.430	39.2	37.6	0.160	3.34	3.27	0.73	2.29	2.24	0.40
5	54.8	101.2	0.026#	91.8	182.5	0.004#	39.8	37.4	0.013#	3.34	3.22	0.55	2.29	2.31	0.56
5.5	64.1	85.4	0.740	104.5	163.5	0.620	37.1	37.2	0.950	3.18	2.95	0.21	2.29	2.30	0.88
6	67.8	83.2	0.490	117.1	151.5	0.260	38.4	36.4	0.150	3.19	2.99	0.35	2.37	2.30	0.13
6.5	61.2	92.2	0.110	135.8	159.4	0.790	38.8	37.6	0.490	3.11	3.05	0.82	2.34	2.26	0.19
7	64.4	79.0	0.640	124.5	134.9	0.960	37.4	35.7	0.300	3.02	3.15	0.62	2.29	2.37	0.31

T: Treatment; C: Control; S: Significance. *: Using independent sample *t* test. #: $P < 0.05$.

lower than that of the placebo group. With regard to AST, there was a significantly lower mean AST level in the vitamin E group on Days 3–5 (Table 3). Mean serum albumin level in the treatment group remained higher than that of the placebo group, with a significant difference seen in Day 2.5 and Day 5 levels (Figure 3 and Table 4). Mean serum cholesterol levels also showed a similar trend from Day 2 to Day 6.5 with vitamin E group showing higher mean level than the placebo group. However, there was no significant difference between the two groups on any of the days (Figure 3 and Table 4). Serum calcium levels in the two groups did not show any distinct pattern.

4. Discussion

The purpose of this study was to determine whether antioxidant properties of vitamin E would be helpful in the management of DF and DHF in children. Despite increasing evidence shows that oxidative stress is an important pathological process in the disease, studies evaluating the efficacy of antioxidant treatment are scarce. The sole interventional study conducted by Vaish *et al.*[18] only focused on the platelet count of adult dengue patients who received vitamin E treatment during the illness. Our study has looked into the effects on all the clinically relevant aspects of the disease including biochemical, haematological and clinical parameters. It has shown that vitamin E treatment significantly improves these parameters in children with DF and DHF.

In this study, WBC remained higher in the treatment group during the course of the illness and the change was significant by Day 6 of the illness. Platelet counts were also higher in the vitamin E group although this did not reach statistically significant levels. This is in keeping with the findings of Vaish *et al.*[18] who demonstrated higher platelet counts in adult patients who were on vitamin E. These findings suggest that vitamin E treatment reduces the bone marrow effects of the dengue virus and also helps in faster recovery.

In the treatment group, serum ALT and AST levels were statistically lower during the course of the illness. As there is direct *in vitro* evidence that oxidative damage occurs in dengue, this shows that antioxidant treatment in the form of vitamin E is liver protective in dengue children.

The crucial event that occurs in DHF is the capillary leak, which causes most of the complications in the disease. Serum albumin level which declines with capillary leak remained higher in the vitamin E group suggesting that there was less leaking. PCV that increases with plasma fluid leaking, was lower in the treatment group. Most

importantly vitamin E treatment reduced the duration of leaking significantly. Therefore this shows that vitamin E ameliorates the process of leaking thus reducing both intensity and duration.

However, our study could not demonstrate any change in the overall disease outcome and the rate of complications. This could be due to the fact that the study was carried out in a small number of patients at an experienced tertiary care centre which maintained a very low complication rate by employing meticulous fluid management and monitoring facilities.

The main limitation of the study was the low sample size. We feel that a larger sample size would have shown statistically significant improvement of some of the parameters, which were monitored. A larger sample would also give an insight into the overall disease outcome in children. Also, we did not have facilities to directly measure the oxidative stress in the study population. Since the study recruited patients who were admitted to hospital, most patients received vitamin E treatment after the first 24 h of the illness. We believe that if treatment is started on day one itself there may be more significant benefits to patients.

Based on our findings, we recommend that vitamin E should be incorporated in the routine management of DF/DHF in children. Furthermore, we hope that this study will form the basis for a multicentre trial in the future, with direct measurements of oxidative stress. This will help in determining the effects of vitamin E treatment on overall disease outcome and in optimising the dose of the vitamin E.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

We thank all participants and their parents for the support given in the study, the medical officers and nursing officers of Professorial Paediatric Unit at Lady Ridgeway Hospital for the management of the patients, the laboratory staff of Lady Ridgeway Hospital for analyzing the blood samples. We also thank George Steuart Health (Pvt) Ltd. for providing dengue IgM/IgG antibody kits (SD Bioline immunochromatography) and Health Care Division of Emerchemie NB (Ceylon) Ltd. for providing the vitamin E (Evion, Merk).

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