""EFFECT OF VITAMIN E & VITAMIN C SUPPLEMENTATION ON THROMBOCYTOPENIA IN DENGUE FEVER" – A RANDOMISED CONTROLLED TRIAL"

BY

REG NO.BM0120006

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Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"EFFECT OF VITAMIN E & VITAMIN C SUPPLEMENTATION ON THROMBOCYTOPENIA IN DENGUE FEVER – A RANDOMISED CONTROLLED TRIAL"**, is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

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LIST OF ABBREVIATIONS

ARDS- Acute Respiratory Distress Syndrome

CEHC- Carboxyethyl Hydroxychroma

CRP- C-Reactive Protein

CYD-TDV- Chimeric Yellow Fever Virus-DENV Tetravalent Dengue Vaccine

DENV- Dengue Virus

DF – Dengue Fever

DHA- Docosahexaenoic Acid

DHF – Dengue Haemorrhagic Fever

DSS – Dengue Shock Syndrome

DV – Dengue Virus

ECF- Extracellular Fluid

GI- Gastrointestinal

GLUTs- Facilitative Glucose Transporters

HDL- High Density Lipoprotein

IL-8- Interleukin 8

LDL- Low Density Lipoprotein

MDA- Melondialdehyde

NSAIDs- Non-steroidal Anti-inflammatory Drugs

PAI-1- Plasminogen Activator Inhibitor 1

PUFA- Polyunsaturated Fatty Acids

RNA- Ribonucleic Acid

ROS- Reactive oxygen species

SVCT2- Sodium-Dependent Vitamin C Transporter 2

URTI- Upper Respiratory Tract Infections

VGEF- Vascular Endothelial Growth Factor

VLDL- Very Low Density Lipoprotein

WHO- World Health Organization

ABSTRACT

"EFFECT OF VITAMIN E & C SUPPLEMENTATION ON THROMBOCYTOPENIA IN DENGUE FEVER"- A RANDOMISED CONTROLLED TRAIL

Introduction:

Dengue virus infection is the most widespread mosquito-borne viral infection in humans and has emerged as a serious global health challenge. When infected with the virus, the immune system gets activated & reactive oxygen species (ROS) are generated. These ROS are removed by anti-oxidants comprised of vitamins (A, C, E), glutathione & enzymes.

Aims & Objective:

To Evaluate the Effect of Vitamin E & Vitamin C supplementation on thrombocytopenia in children between 5yrs to 18yrs with dengue fever.

Methods:

A Randomized Controlled Trial was conducted at KLE Dr. Prabhakar Kore Hospital and Medical Research Centre for one year. Randomly assigned using statistical software R version to either of 2 groups. Children between the ages of 5-18 years, who were diagnosed NS1AG Positive or dengue IgG & IgM Positive were included. Patients who have severe GI bleeding manifestations were excluded.

Group 1 (Experimental): Receive Vitamin E & Vitamin C Supplements for 5 days.

Group 2 (Control): Receive standard treatment only.

Results & Conclusion:

In the experimental group, there is a 269% increase in platelet count from the first day to 5th day. In the Control group, there is a 195% increase in platelet count from the first day to 5th day. Thus Vitamin E & C have shown beneficial effect on thrombocytopenia in dengue fever and results in faster increase in the platelet counts.

KEYWORDS: Vitamin E, Vitamin C, Dengue fever, Platelet count

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INTRODUCTION

Dengue is a commonest arboviral infection globally, particularly in tropical countries. It manifests from a self-limiting illness to death. The majority of mortality due to dengue occurs with prolonged shock which resulted from third space loss and bleeding.[1]

There have been four different serotypes of dengue virus discovered so far (DENV1, DENV2, DENV3, and DENV4). Each serotype has a variety of genotypes, including three for DENV1, two for DENV2, four for DENV3, and four for DENV4. The virus possesses three structural protein genes (which code for a core protein, a membrane-connected protein, and an envelope protein of the nucleocapsid) and seven non-structural protein genes; however, only the NS1 antigen is known to interact with the host immune system, and the functions of the other non-structural proteins are not very well understood.[2]

Patients with dengue virus infection may experience platelet destruction that causes the release of VEGF, which causes third-space fluid loss, such as pleural effusion and ascites.[3,4]

Reactive oxygen species (ROS) are produced when the immune system is stimulated after a viral infection. Antioxidants, which are made up of vitamins (A, C and E), glutathione & enzymes, eliminate these ROS (superoxide dismutase & glutathione peroxidase). Reduced glutathione antioxidant activity and increased superoxide dismutase activity in dengue patients were suggestive of increased oxidant activity and reduced anti-oxidant capability. Dengue shock syndrome displayed the highest levels of reactive oxygen species. It is thought that oxidative stress contributes to liver and tissue damage in dengue infection. Consequently, antioxidant properties may have a role in management.[5]

Platelets are equipped with a transporter for vitamin C. (SVCT2). Platelets are equipped with a transporter that enables them to actively carry vitamin C into the cells, where it may affect the oxidative stress levels. It has been shown that supplementation with it may boost the immune system, avoid harm to cells that are involved in the innate immune response, and heal tissues that have been injured by the host's defenses.[6]

Thrombocytopenia, which results from the bleeding symptoms of dengue fever, is the main danger. It is unclear how dengue fever causes thrombocytopenia to develop, however increased peroxidation and vitamin supplementation may play a part. Vitamin E has antioxidant property and Vitamin C stimulates immune system function and may help in these cases.[7]

As a consequence of this, there is increasing evidence that chronic stress is a significant pathogenic mechanism in the severity of dengue, despite the fact that there is no data on the efficiency of anti-oxidant treatment. The present study was to look at the effect of supplementation of vitamin E and vitamin C among children with dengue fever with thrombocytopenia.

OBJECTIVES

To evaluate the effect of vitamin E and vitamin C supplementation on thrombocytopenia in children between 5 years to 18 years with dengue fever.

REVIEW OF LITERATURE

Dengue fever:

Epidemiology:

According to WHO, dengue is considered a global threat in tropical and sub-tropical countries. In south-east Asian countries, particularly India during the last decade, dengue fever has become a serious public health problem.[1]

The spread of dengue fever from urban to rural areas has contributed to a thirtyfold rise in the disease's prevalence during the previous fifty years. It is estimated that 50 million individuals get infected every year, and 2.5 million people are at risk in areas where the disease is prevalent. The unplanned urbanisation, overcrowding, population expansion, ineffective vector management, and lack of access to appropriate healthcare services have been blamed for the rise in the incidence of dengue infection rates and death that has occurred during the previous ten years.

People of all ages were affected by the illness, and it did not show any racial or ethnic biases in its spread. The severity of the illness displayed a bimodal distribution, with the elderly and babies consistently having a higher fatality rate than other age groups. The risk of severe dengue fever is higher in children than it is in adults. This may be due to the fact that adults have a more powerful immune response and more co-morbidities, both of which contribute to the severity of the illness. The World Health Organization (WHO) has determined that Southeast Asia, including India, is a dengue-endemic region.[1,8,9] Every state has a problem with dengue fever, with the exception of the islands that make up Lakshadweep.[2]

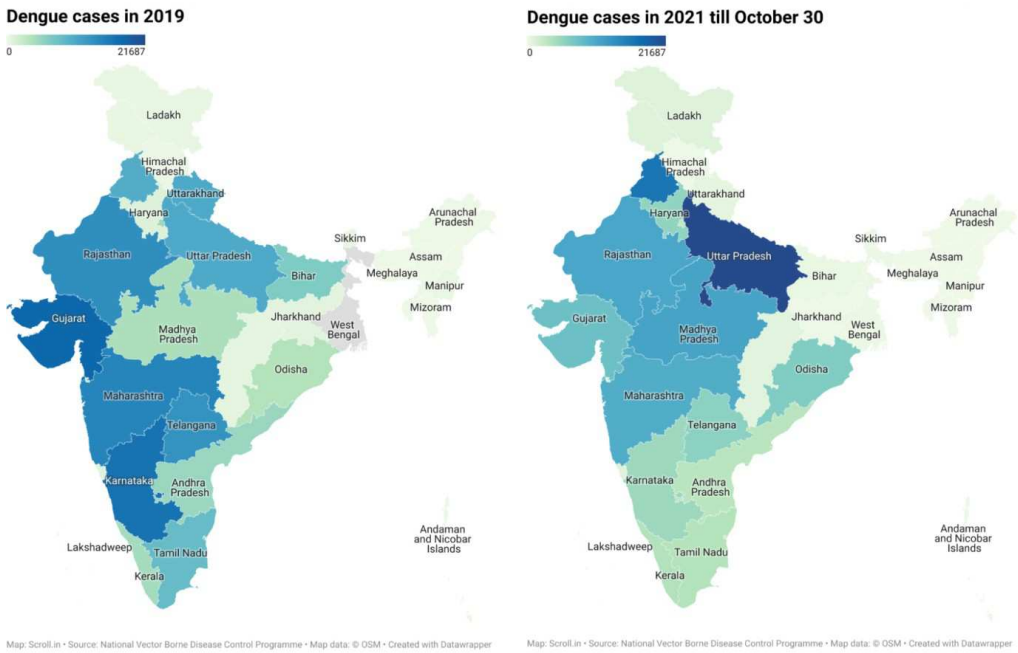


Figure 1: Incidence of dengue cases in India 2019 & 2020.

In India, it has been claimed that there have been cyclical outbreaks or epidemics, with an increase in infection happening during the monsoon seasons. During the wet season, stagnant water may gather in outdoor reservoirs such as plastic cups, coconut kernels, mortars, buckets, and the like, all of which can serve as a breeding ground for mosquitoes. In metropolitan locations, the containers that served as breeding grounds for mosquitoes included things like refrigerator trays, air conditioner trays, flower vases, containers for holding drinking water, and so on. Seasonal outbreaks have been occurring in India with an increased number of symptomatic cases, particularly with increasing morbidity and mortality in various parts of India including Delhi, UP, Karnataka, Maharashtra, and Tamil Nadu. In addition, the number of symptomatic cases has been rising. In South India, the most recent epidemic occurred in 2017, mostly in Chennai and the districts around it.[10,11]

Agent factors:

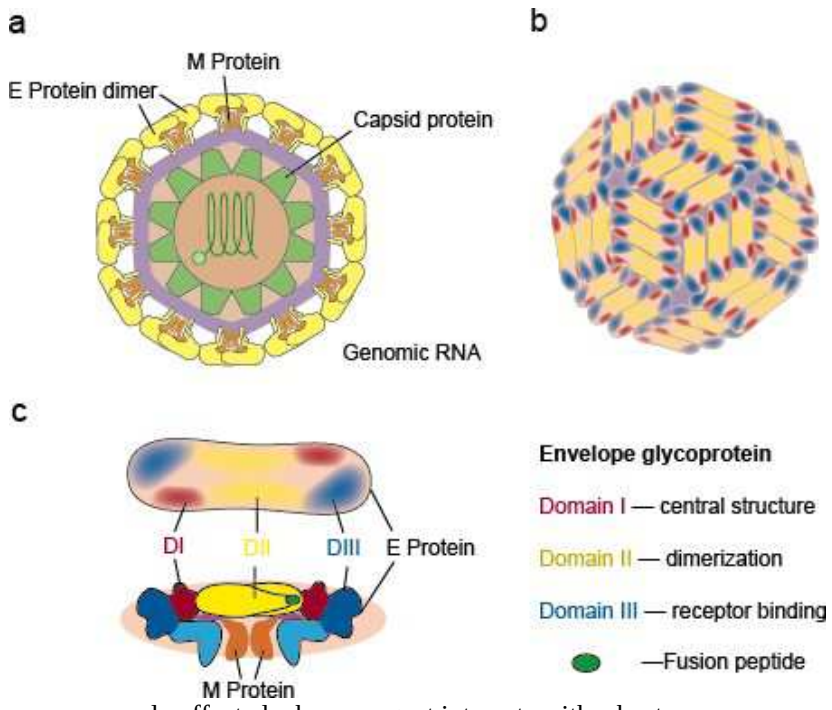
The Dengue virus:

Dengue viruses, which act as the disease's agent, belong to the genus Flavivirus. It is 50 nm in size and comprises ssRNA. There are 4 strains of the dengue virus viz, Dengue virus-1, 2, 3, 4. Immunity to the viral serotype is conferred upon infection with any one serotype. Despite the antigenic similarity of all 4 serotypes, cross-immunity against dengue viruses only develops a few months following infection.

In the four dengue virus varieties, Dengue virus serotypes include dengue virus 1-4. It is part of a phylogenetic group and has different nucleotide sequences from one another. Rather of being related to other flaviviruses, these serotypes are strongly connected to one another. It creates an independent antigenic complex.

The DENV genome is composed of seven non-structural NS-protein genes in addition to three structural protein genes, which code for the enveloped protein-E, Protein-M, and nucleocapsid of core protein. The NS1 gene is the one that is most usually used. There are several functions that non-structural proteins play that are not completely known. Non-structural protein 1, on the other hand, was shown to interact with the host immunity and was shown to initiate immunological responses in T cells. Patients with DENV infection have detectable non-structural protein1 levels in their blood. These are used to identify the infection as a diagnostic sign.[14]

Figure 2: Structure of dengue virus



Most cases of dengue are subclinical. It is still unclear what specifically makes some patients more severely affected when an agent interacts with a host.

Vector:

The bite of an infected female Aedes mosquito is the vector by which dengue viruses are transmitted from one person to another. *The primary vector in India's metropolitan areas is Ae. aegypti*, Ae. albopictus, however, is also a problem in several states. *In other nations, Ae. polynesiensis and Ae. niveus have also been implicated as secondary vectors*.

The female mosquito lays one egg on wet surfaces. When conditions are familiar, the adult of the Ae. aegypti life cycle emerges seven days after the aquatic stages. At cold temperatures, it can take many weeks to grow. When the eggs are subjected to water, they have the ability to withstand drying for more than a year and still hatch within a single day. It is also a significant issue for dengue prevention and management.[15,16]

The disease's spread and the life cycle depend on climatic conditions, particularly temperature and rainfall. Ae. aegypti and Ae. albopictus have average lifespans of thirty days and eight weeks, respectively. During the rainy season, the probability of viral transmission is higher than usual. It feeds throughout the day and is capable of flying up to a distance of 400 metres. In light of the fact that there is now no treatment or vaccination available specifically for DENV, it is very crucial to manage the vectors that spread illness.

Aedes almost always reproduces in domestic water sources, which can be located in or close to homes, overhead tanks, desert coolers, unused tyres, coconut shells, industrial and domestic junk, water storage containers, water reservoirs, construction sites, disposable cups, unused grinding stones, and other similar places. Ae. Albopictus prefers natural larval habitats which include bamboo stumps, coconut shells, leaf axils, tree holes, and latex collecting cups in plantation crops. In more recent times, evidence of its replication in domestic situations has also been gathered.

Environmental factors:

The Aedes aegypti mosquito adapts to different water storage conditions. The lifespan of Ae. aegypti may be affected by both temperature and humidity. It feeds on human beings and seeks for dark, cool areas to relax in. It is most likely to survive if the temperature is between 16 and 30 degrees Celsius and the relative humidity is between 60 and 80%. Another limiting element for the dispersion is altitude. It can only be done at elevations of up to 1000 feet above sea level. Ae. aegypti's rural spread is a relatively recent phenomenon linked to cultural and behavioral changes in rural areas.

Host factors:

Humans and several other species of lower primates are infected by the virus. It affects people of all ages and genders. Secondary dengue infection, including antibodies acquired passively in neonates, is one of the risk factors for dengue hemorrhagic fever (DHF). Travel to dengue-affected regions is the single most significant risk factor for getting dengue.

If the fever lasts for more than two weeks and the patient has a history of travelling, however, it is very improbable that the patient has dengue fever. The dengue virus may spreads to others, if an infected individual migrates there while in viremia. The virus's geographic spread had been mainly by individuals relocating from endemic to non-endemic areas.[18]

Transmission:

When a person's blood is taken during the viremia, the female Ae. aegypti typically contracts the dengue virus (acute febrile) phase of dengue fever. After an 8 to 10 day incubation period, the mosquito contracts the disease. When an infected female mosquito bites, it spreads. Following a 4 to 7 day incubation period, dengue fever starts suddenly. There is also evidence of vertical transmission of the dengue virus, in which infected female mosquitoes pass the virus on to the next generation of their species.

There are additional reports of dengue spread through organ transplantation and blood transfusion.

Pathophysiology:[20]

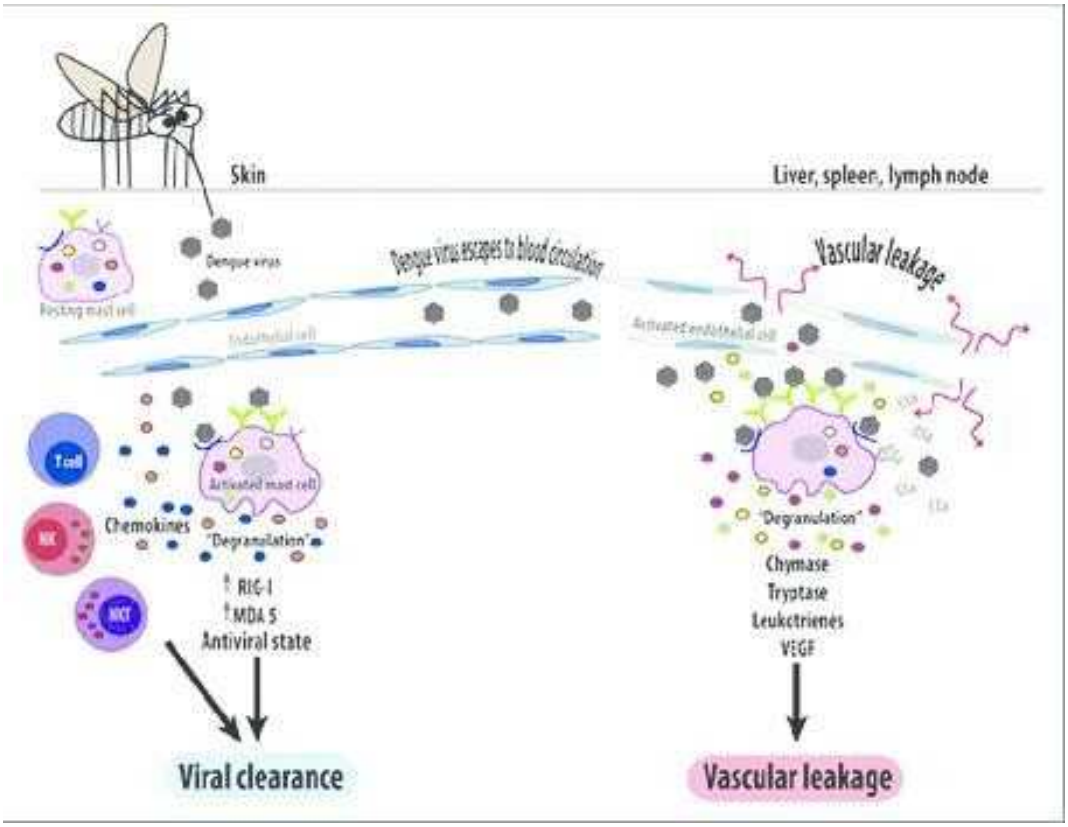


Figure 3: Pathogenesis of dengue infection

Clinical features:

Dengue patients may be asymptomatic or symptomatic. Clinical manifestations vary from undifferentiated fever to DHF & DSS. The clinical symptoms are determined by a number of criteria including the virus strain, the immunological condition of the host, the age of the patient, and whether the infection was primary or secondary. Infection with a single dengue serotype confers protection against all other dengue serotypes throughout a person's whole life.

Patients with primary dengue may have a fever ranging from low to severe. It is difficult to distinguish from other infectious viral diseases. During episodes of fever or convalescence, the maculopapular rash may or may not manifest itself. It might be difficult to

differentiate the symptoms of dengue fever from those of other illnesses. It's possible that there won't be any signs of capillary leaking.[21]

Severe dengue:

The majority of infected individuals are asymptomatic, however those who are symptomatic may present with an undifferentiated fever and mild to severe symptoms. Some dengue virus patients exhibit severe symptoms, such as low pulse volume, plasma leakage, hemorrhage, and organ involvement. Four grades have been established for DHF. DF & DHF grade I and II without considerable bleeding are possible in non-severe instances. DHF III and IV can indicate severe dengue. When infected with the dengue virus, some people exhibit severe symptoms such as low pulse volume, plasma leakage, hemorrhage.

Symptoms and signs:

The following signs and symptoms can be used to gauge the severity and course of DF/DHF/DSS:[23]

Table 1: Signs and symptoms:

Recurrent vomiting
Abdominal pain or discomfort
Palpitation, breathlessness
Pleural effusion/ ascites/ gall bladder edema
Hepatic dysfunction
Decreased urinary output
Minor bleeding from different sites
Narrow pulse pressure
Rapid pulse
Hypotension
High HCT
Cold clammy extremities
Rapid fall in platelet count

Expanded dengue syndrome:

Uncommon manifestations of DF/Dengue Hemorrhagic fever are a condition frequently linked to co-morbidities and co-infections. The following clinical signs and symptoms have been seen in extended dengue syndrome:[24,25]

Table 1: Clinical features:

System	Unusual or atypical manifestations
CNS	Encephalopathy, encephalitis, febrile seizures, I/C bleed
GI	Acute hepatitis/ fulminant hepatic failure, cholangitis, acute pancreatitis
Renal	Acute renal failure, haemolytic uremic syndrome, acute tubular necrosis
Cardiac	Cardiac arrhythmia, cardiomyopathy, myocarditis, pericardial effusion
Respiratory	Pulmonary edema, ARDS, pulmonary hemorrhage, pleural effusion
Eye	Conjunctival bleed, macular hemorrhage, visual impairment, optic neuritis

Dengue among pediatric-age patients:

Dengue illness affects people of all ages, with children being the age group that has been proven to be most affected. Age groups in children are also particularly vulnerable to morbidity & mortality. In recent papers, it has been noted that dengue infection is highly prevalent in children, adolescents, and adults.

The Neonatal dengue infections:

According to the findings of a number of studies, the vertical transmission of dengue from pregnant women to their unborn children ranges from 1.6 to 64 percent. Influence of dengue fever on expectant mothers and foetusesin addition, babies should be subjected to a comprehensive examination to screen for capillary leaks and a tendency to bleed. Infected newborns may exhibit symptoms such as fever, thrombocytopenia, petechial rash, and hepatomegaly. These symptoms can range from mild to severe, including gastrointestinal bleeding, pleural effusion, circulatory failure, and extensive intracerebral haemorrhage. It is not clear if the clinical appearance of the newborn child is connected to the severity of the mother's sickness, her immunity against dengue, or the way by which the baby was delivered. Even while a maternal infection during the peripartum period may increase the probability of a symptomatic infection in the newborn, the timing of the illness in the mother may also be crucial. The passive transmission of maternal dengue antibodies to the unborn has been shown to have an effect on the severity of the disease's course in certain cases. [Citation needed] It is possible for pregnant women who have dengue infection to have antibodies to the DENV virus. These antibodies may cross the placenta and infect the foetus with a severe form of dengue. It is possible that the first symptoms are those of bacterial sepsis, birth traumas, or other newborn illnesses.[16]

Dengue infection among infants:

For newborns, the dengue virus can have a variety of effects. It can range from an infection with no symptoms to a mild or clinically significant serious condition that affects older children. Infants between the ages of 4 and 9 months are the main age range for severe dengue.[16]

Clinical features:

Infants with DF frequently have a high fever lasting 2–7 days, similar to adult children. Infants with dengue are more likely to experience URTI, GI manifestations, and febrile convulsions than older children. When a newborn is febrile, it is frequently difficult to distinguish between dengue and other prevalent infections such pneumonia, measles, meningoencephalitis, rotavirus infection, etc.

The majority of dengue newborns will see an increase in plasma leakage around the defervescence (3-6 days of sickness), along with rising hematocrit levels. Clinical plasma leakage lasts between 24 and 48 hours. During this critical stage, the clinical characteristics of newborns with dengue infection become more. Petechiae, mucosal membrane bleeding, and GI bleeding are present. In the newborns with dengue infection, hepato-splenomegaly is reported in roughly 10% of cases. When a considerable amount of plasma leaks, low blood pressure will be recorded.

Dengue fever:

An acute febrile illness of 2-7 days duration with two or more of the following manifestations:

Headache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic manifestations.

Dengue hemorrhagic fever:

A case with clinical criteria of dengue fever

+

Hemorrhagic tendencies evidenced by one or more of the following:

1. Positive tourniquet test
2. Petechiae, ecchymoses or purpura
3. Bleeding from mucosa, gastro-intestinal tract, injection sites or other sites

+

Thrombocytopenia - <1,00,000 cells per cumm

+

Evidence of plasma leakage due to increased vascular permeability, manifested by one or more of the following:

1. A rise in average hematocrit for age and age >20%
2. A more than 20% drop in hematocrit following volume replacement treatment compared to baseline
3. Signs of plasma leakage

Dengue shock syndrome:

All of the preceding criteria for DHF, in addition to indications of shock, which includes hypotension, cold and clammy skin, and restlessness. Narrow pulse pressure (less than 20% mm Hg) is another indicator of shock.

The natural course of dengue infection

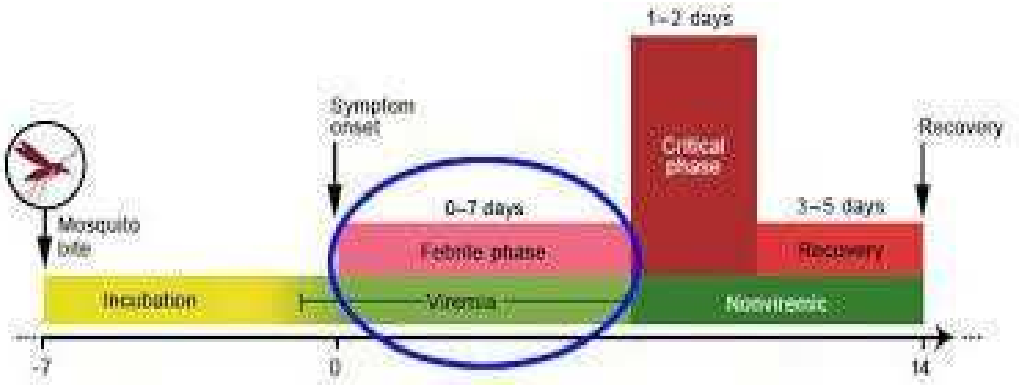


Figure 4: Natural course of dengue infection

The Febrile phase:

Dengue fever often begins with an increase in temperature that lasts 2–7 days, may be biphasic, and is accompanied by flushing, headache, and rash. Pain in the retro-orbital region, muscles, joints, or bones is possible. The rash appears after the third or fourth day of fever and is frequently visible on the face, neck, and other parts of the body and disappears in the final stage of the feverish phase. Over the upper and lower limbs, there may be a discrete cluster of petechiae. In rare circumstances of co-morbid illness, dengue fever with hemorrhagic form may be found.[27,28]

Critical phase:

Patients with DF/DHF typically enter the critical phase 3 to 4 days following the beginning of fever. Plasma leakage and significant hemoconcentration are observed at the critical stage, and patients may experience hypotension. Plasma leakage and abnormal hemostasis cause shock, haemorrhage, and fluid buildup in the pleural and abdominal cavities. In DHF/DSS, high morbidity and mortality rates are typical. This stage will involve the involvement of the organs and a disturbance in metabolism. Plasma leakage typically lasts between 36 and 48 hours.[29,30]

Recovery phase:

The extracellular fluid that was lost as a result of capillary leakage is reabsorbed into the circulatory system during the recovery phase, and signs and symptoms start to improve. Usually appearing after 6-7 days of fever, this phase lasts for two to three days. Some of the patients who need specialized medical treatment due to severe shock, organ involvement, and other concerns may take a longer length of time to recover completely. If the patient's fluid replacement is not carefully optimized, fluid overload could result in pulmonary edema.[31]

Management:

DF:

DF is managed symptomatically and supportively**:**[33]

1. Antipyretics. Aspirin/NSAIDs should be avoided. Paracetamol is preferable
2. In order to keep the temperature below 38.5oC, use cold or tepid sponging.
3. It is advised to stay in bed throughout the acute period.
4. 4. A kid who is vomiting excessively or sweating excessively should undergo treatment with an oral fluid and electrolyte replacement solution.
5. After they become afebrile, they need to be watched for the emergence of problems for 1 to 2 days.

Management of DHF:

Grade I and II:

Hospitalization is recommended for anyone who has dengue fever, has thrombocytopenia, high hemoconcentration, and exhibits symptoms like stomach pain, epistaxis, black tarry stools, bleeding gums, etc. in order to check for signs of shock. The transition from a febrile to an afebrile phase, which often takes place after the third day of illness, is when shock is most likely to develop. The increase in

hemoconcentration implies plasma leakage, which is related to proper fluid management. The care for Grade III or IV DHF or DSS should be implemented if the patient had low blood pressure, a decrease in urine output, or other signs of shock despite the medication.[34]

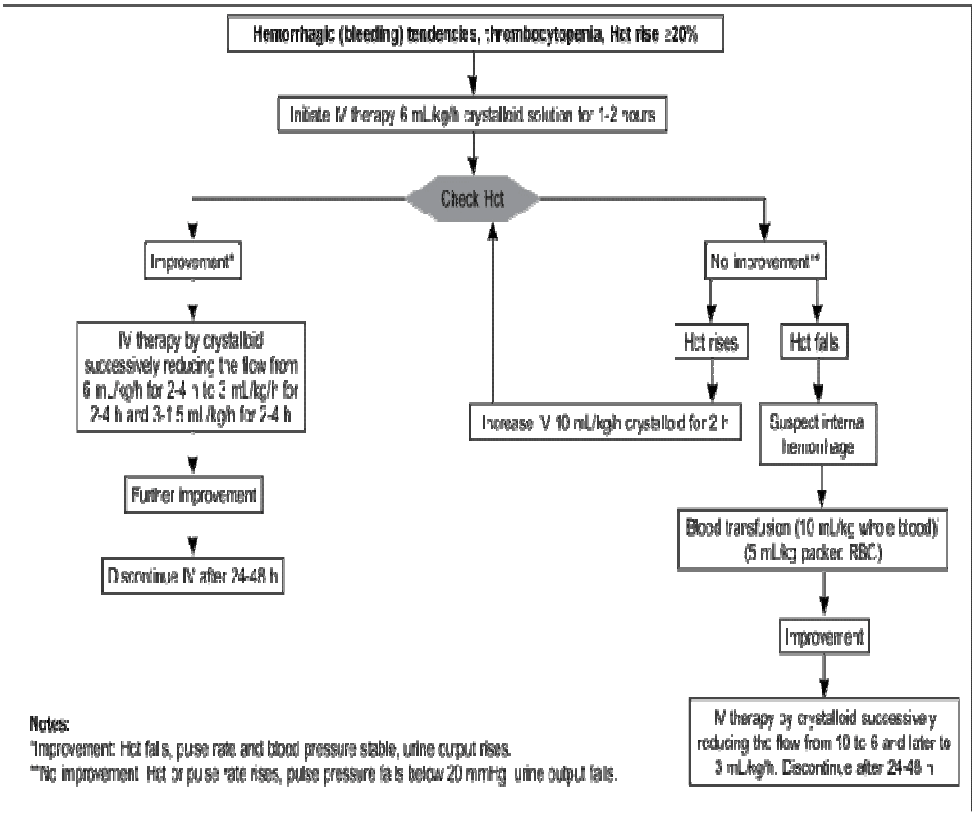


Figure 5: Volume management among patients with DHF

Following hospitalisation, pertinent tests and vital signs should be evaluated, and hydration therapy should start right once. If the patient has previously had around 1000 ml of intravenous fluid, the fluid should be changed to a colloidal solution, preferably Dextran40. If the patient's hematocrit continues to decline, a new whole blood transfusion of 10–20 ml/kg/dose should be delivered.

Internal bleeding should, however, be suspected in cases with fluid-refractory shock if the hematocrit continues to drop. Estimating the amount of internal blood loss may be difficult when hemoconcentration is present. All patients who are in shock should have whole blood given to them in moderate amounts, at a rate of 10 millilitres per kilogram per hour. This is a normal precaution. Every patient who is in shock has to have extra oxygen administered to them.[35]

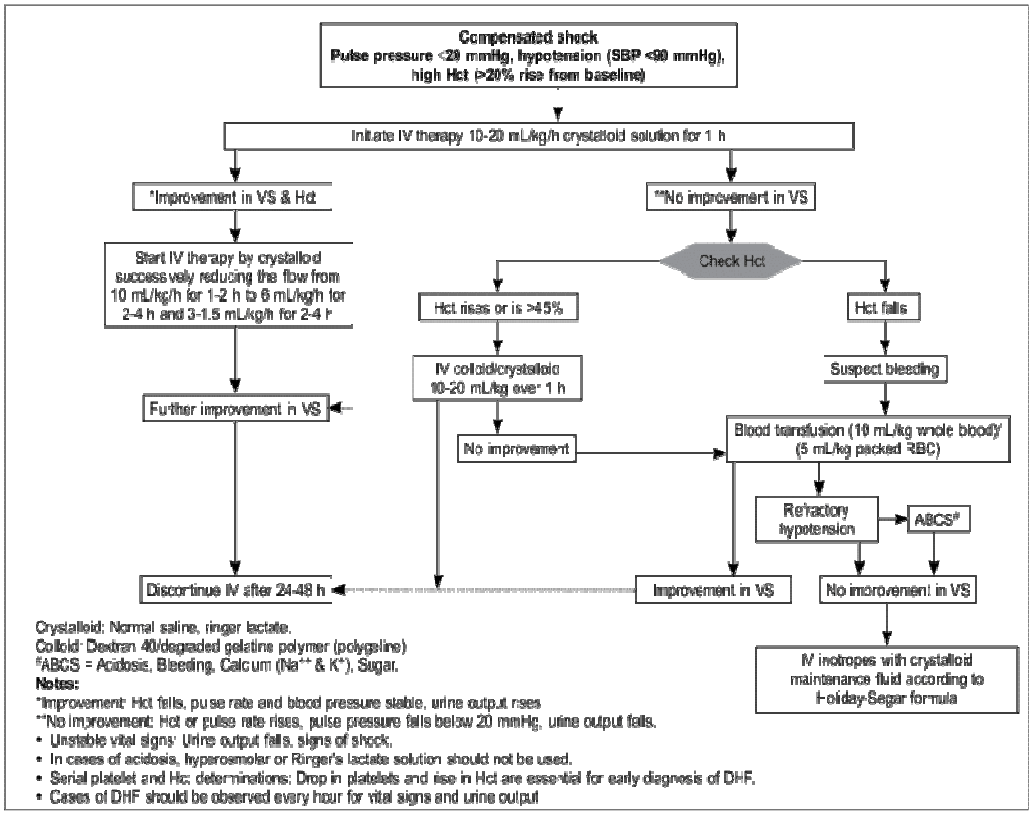


Figure 6: Volume management among patients with DHF grade III

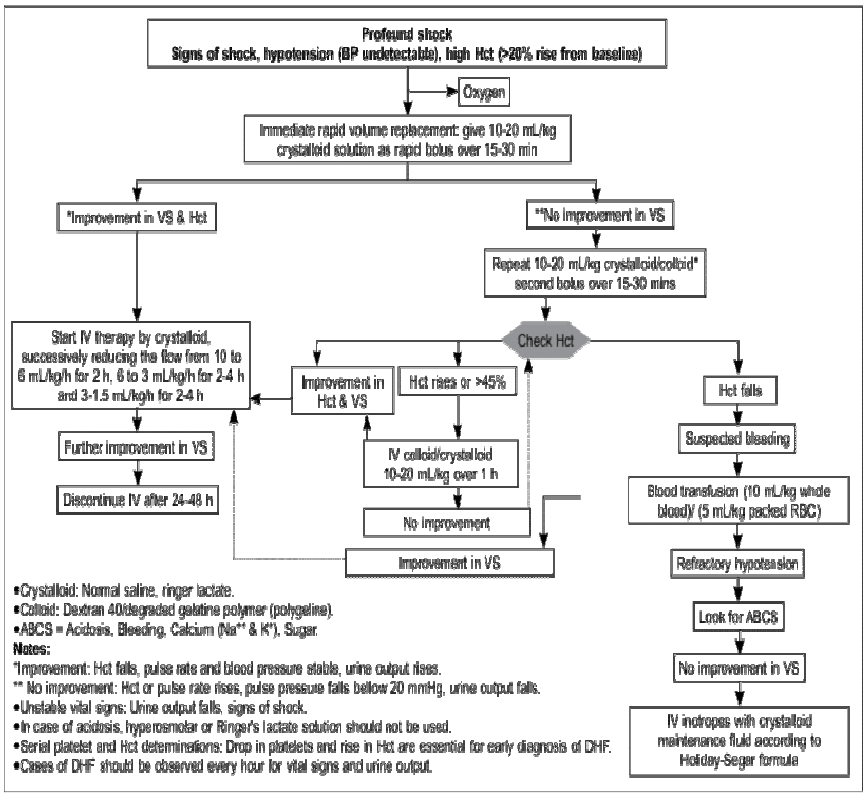


Figure 7: Volume management among patients with DHF grade IVDengue vaccine:

Currently, there is no licenced dengue vaccine on the market. The creation of a dengue fever vaccine is currently the subject of numerous experiments worldwide. Children from five Asian nations have completed phase III trials for a recombinant, live attenuated tetravalent dengue vaccine called CYD-TDV, which may show promise in the near future for avoiding dengue infection.[36]

Vitamin C:

In addition to being a necessary micronutrient, the human body uses vitamin C for a variety of different biological processes. The majority of mammalian species use gulono-gamma-lactone oxidase as the final step in their metabolic pathway to create ascorbic acid from glucose in the liver. However, as a result of a mutation in the gulono-gammalactone oxidase gene, monkeys and guinea pigs are fully reliant on the vitamin C that is provided to them via their diet. As a consequence of this, scurvy may develop in people if they do not get enough vitamin C in their diets. Scurvy is a sign of vitamin C deficiency.[37,38]

Recommended daily intake:

The Recommended daily is in the range of 75 to 110 mg/day.[39]

Structure:

Vitamin C is the L -enantiomer of Ascorbate, with chemical formula C6H8O6. Vitamin C can assume 3 redox forms in the body. In healthy physiological settings, the majority of the vitamin C in the body exists as the ascorbate anion. 40

From a structural perspective, it is also one of the few molecules with a carbon-3 hydroxyl group that has a pKa of 4.2, making it totally dissociable at neutral pH.[41]

It is possible that all of the physiological effects of ascorbate are caused by the electrons that are present in its molecule. Because it may donate electrons, vitamin C is considered to be a reducing agent. Be a result of the ability of the molecule's electrons to lower the number of oxidised species, vitamin C is often referred to as an anti-oxidant. However, this nomenclature isn't quite accurate in all respects. Vitamin C has the ability to remove electrons from metals such as iron and copper,

which ultimately leads to the production of reactive oxygen species (ROS) as a byproduct of the synthesis of superoxide and hydrogen peroxide. As a consequence of this, ascorbate may on occasion generate oxidants.[42]

Vitamin C can be found in trace amounts in fresh meat, fish, green leafy vegetables, fruits, and germinating pulses.

One of the richest sources, both in its fresh and dried forms, is amla.

Another inexpensive yet abundant source of vitamin C is guava.

Metabolism:

Synthesis:

Numerous animals are capable of producing their own vitamin C. Every plant species that has been investigated to this point also produces vitamin C. Yeasts produce D-dehydroascorbate, which is a C5 derivative of ascorbate. However, mammals, plants, and fungi all manufacture vitamin C in their own unique ways due to their own metabolic processes. [43] The production of vitamin C in animals has been well investigated in Sea Lamprey, leading researchers to hypothesise that it first appeared in early vertebrates. However, throughout time, the capacity to biosynthesize vitamin C was lost in a number of animals, including certain kinds of fishes and birds, as well as bats, Guinea pigs, and primates, including humans. Vitamin C is produced in the kidneys of fish, amphibians, and reptiles, while it is made in the liver of mammals.[44]

Absorption and transport:

Because the human body is unable to produce ascorbic acid on its own, this vitamin is considered to be a necessary micronutrient. Ascorbic acid and dehydroascorbic acid are the oxidised forms of vitamin C that humans receive from food in order to meet their vitamin C requirements.[45]

Vitamin C uses 2 groups of transporters:[45]

• Sodium-dependent transporters (SVCT) 1, 2, which are specialised transporters for ascorbic acid and are responsible for its entry into the cell The small intestine epithelium and the proximal convoluted tubule of the kidneys both contain SVCT 1, whereas other tissues include SVCT 2.

• Glucose transporters, also known as GLUTs, which are responsible for bringing the product of ascorbate oxidation, DHA, into the cells. As soon as it enters the cell, DHA is quickly converted to ascorbate. Ascorbate recycling is the name given to this process, which involves the intracellular transfer of vitamin C.

The SVCT1 transporter is responsible for the absorption of vitamin C from the small intestine. There is a possibility that some ascorbic acid will be oxidised in the gut to DHA, which will then be transported via GLUT. Following absorption, the water- and fat-soluble vitamin C travels from the circulation to the extracellular fluid and is dispersed there (ECF). [45] The majority of vitamin C is taken up by tissues through the SVCT 2 transporter. Vitamin C levels in tissues are dependent on the concentration gradient, which in turn is determined by how much of the vitamin is taken in.

Tissue concentrations are usually in milli molars and far higher than the concentration required for its action as a coenzyme. The major portion is in the liver, brain, and Kidneys.[45]

Other than Saliva, RBC are the only body compartment that has lower Vitamin C levels as compared to plasma. RBCs obtain it via a DHA pathway (ascorbate recycling) since mature RBCs do not contain SVCT2. There is also constant efflux of Vitamin C from cells to plasma to maintain equilibrium. The mechanism of Vitamin C efflux is unknown. SVCT 1 is also responsible for the re-absorption from the renal proximal convoluted tubule.

Metabolism:

More than 99% of ascorbate formation in the body takes place while the body is functioning normally from a physiological standpoint. It is possible for it to donate two electrons in a row, which would result in the formation of ascorbate radical and DHA, respectively, from the double bond that exists between carbons 2 and 3(4). 2, 3 diketogulonic acid is produced by hydrolysis and irreversible ring rupture of DHA. The metabolic byproducts of 2, 3-diketogulonic acid include oxalate, threonate, and maybe xylose, xylonic acid, and lynxonic acid; however, only oxalate is therapeutically significant. DHA can be converted back to ascorbic acid either instantly through enzyme-dependent pathways or gradually through glutathione to ascorbate radical and ascorbic acid. Vitamin C's ability to donate electrons explains its range of physiological effects.[46]

The Role of vitamin C in the management of capillary leaks:

Vitamin C has been investigated for use in a variety of disorders linked with a breakdown of the capillary barrier, which may result in plasma loss. A newly developed treatment for adults, high doses of ascorbic acid are given to patients suffering from sepsis and burns. In vitro research has shown the processes underlying ascorbate's impact on various disorders.

In endothelial cells, ascorbate lessens oxidative stress by lowering the generation of reactive oxygen species. Respiratory Oxygen The endothelial permeability is increased by species, which results in edoema and contributes to organ dysfunction. Through a number of mechanisms, ascorbate can thicken the endothelium barrier. There haven't been many research done on how a high dosage of ascorbic acid may help avoid capillary leaks, therefore we don't know much about it.

There has been no investigation on whether or not ascorbic acid plays a part in dengue capillary syndrome. In the next series of investigations, researchers investigated how effective ascorbic acid was at avoiding endothelial dysfunction and capillary leakage in animal models as well as in human patients with sepsis and burns. [47]

Parenteral delivery of vitamin C reduced the need for inotropes as well as the demand for mechanical breathing. Vitamin C was hypothesised to have a significant role as a co-factor in the production of endogenous vasopressors, which was the theorised mechanism. Ascorbic acid is a necessary co-factor for the endogenous production of adrenaline, dopamine, and other related substances. [48]

Because severe dengue fever often manifests itself with shock, which is clinically comparable to septic shock, the injection of vitamin C may lessen the need for intravenous fluids and vasopressors.[49,50]

Vitamin E:

In the year 1922, it was found that a fat-soluble food component was required for the prevention of foetal death and sterility in rats. This discovery came about as a result of an experiment. When it was first found in 1936 in wheat germ oil, it was given the names "Factor S" and "ant sterility factor," but now it is more often referred to by its scientific name, vitamin E. The verb "to give birth to offspring" is conveyed by the Greek words tokos and pherein, which are the origins of the word "tocopherol," which is the name given to vitamin E. Tocopherols are known to exist in a number of different forms; nevertheless, the term "vitamin E" is often used to refer to any combination of tocopherols that are physiologically active. This is because vitamin E may be found in a number of different foods.[51]

Structure:

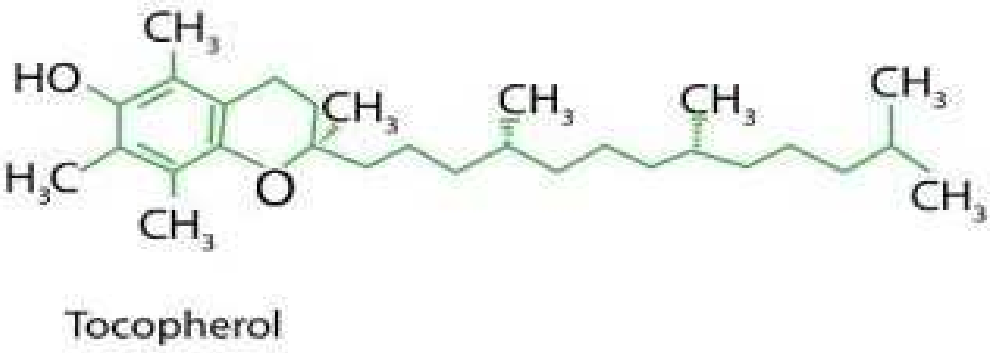


Figure 8: Structure of vitamin E Sources:

Nuts, wheat germ, sunflower seeds, whole grains, seafood, and green leafy vegetables are examples of healthy food.

Commercial vitamin E supplements fall into a number of different categories, including:

The most popular supplement form of fully synthetic vitamin E, "dl - alpha tocopherol," is often the acetate ester.

Less fractionated "natural mixed tocopherols," a synthetic variant of the "natural source" vitamin E ester.

As a result of their susceptibility to oxidation, tocopherols and tocotrienols are often acetylated or succinylated in industrial processes designed to produce vitamin E. Enzymes found in the pancreas hydrolyze the resulting esters, which results in the production of free tocopherols that are physiologically active.

Pharmacokinetics:

Absorption:

The enterocytes in the small intestine absorb vitamin E through passive diffusion. After that, chylomicrons that enter the systemic circulation incorporate vitamin E. Here, lipoprotein lipase, which is found in the capillary endothelium, is responsible for lipolyzing the majority of the chylomicrons. The liver receives the leftover chylomicrons as a result. Here, vitamin E is released in VLDL and repackaged. After VLDL is released into the plasma, vitamin E is transferred by lipolysis to HDL and LDL, which then transport vitamin E to the peripheral tissues.[52]

Metabolism:

By being oxidised by CYP4F2 and CYP3A, vitamin E is converted to carboxyethyl hydroxychroma (CEHC), which is then expelled after being glucuronidated and sulfurized. These metabolites are eliminated by the bile and urine. The faeces contain unmodified excretions of unabsorbed vitamin E.[52]

Storage:

Vitamin E has a finite amount of storage. Vitamin E is only temporarily stored in modest amounts in the liver. Vitamin E is also stored in the adrenal gland and adipose tissue. Vitamin E is gradually accumulated in adipose tissue and then released over time. The simon metabolites—tocopheronic acid and tocopheronolactone—that result from the extensive metabolism of vitamin E in the liver are known as simon metabolites. The urine excretes both metabolites as glucuronides or sulphates.[52]

Role in dengue:

Vitamin E is largely found in the phospholipid bilayer of cell membranes where it has a significant biological role in preventing free radicals from oxidising polyunsaturated fats and other components of the cell membranes.

A process known as lipid peroxidation involves the oxidative decomposition of omega 3 and omega 6 polyunsaturated fatty acids (PUFA) found in membrane phospholipids. This process results in the formation of lipid hydroperoxides as well as aldehydic end products such as melondialdehyde (MDA) and 4 hydroxynonenol. This process has the potential to disturb the structure and function of cells, and as a result, it plays a role in the genesis of a wide variety of disorders. Free radicals have a role in both the beginning stages of lipid peroxidation as well as its progression. A chain reaction may be started when the hydrogen is removed from the PUFA:H molecule by a free radical known as R*. Vitamin E is largely found in the phospholipid bilayer of

cell membranes where it has a significant biological role in preventing free radicals from oxidising polyunsaturated fats and other components of the cell membranes.

More than 60 cytotoxic end products could be created by the aforementioned autocatalytic process. But in biological systems, vitamin E, which is present in the cell membrane, is more likely to stop the peroxidative cascade.

Vitamin E loses its ability to act as an antioxidant and becomes a relatively non-reactive free radical (tocopheroxyl radical) when the hydrogen is removed. It is also unable to target nearby fatty acids. Vitamin C regenerates tocopherol from tocopheroxyl radicals while conserving vitamin E..

Other functions:

In addition to its job as an antioxidant, vitamin E may also be crucial for other biological advancements. These consist of:

- The structural functions in maintaining the integrity of cell membranes.
- Vitamin E has anti-inflammatory qualities because it reduces the production of arachidonic acid, which in turn inhibits the processes of cyclo-oxygenase and lipoxygenase. With sustained therapy, it has been found to lower the release of chemokines that promote inflammation, such as IL-8 and PAI-1, and to lessen the adherence of monocytes to the endothelium. In addition, the release of chemokines that promote inflammation has been shown to decrease. In addition to this, it reduces the amount of CRP.
    - Through modulation of protein kinase C, intercellular signalling and cell proliferation are controlled.
- Nitric oxide has been linked to central pain processing and is suppressed in analgesic action, which has a central analgesic impact. NO lowers nociceptors'

thresholds, lowers the threshold in the periphery, and makes it easier for nociceptive signals to travel through central pathways. Protein kinase C, a crucial component of signal transduction brought on by neurotransmitters and cellular stimuli, is inhibited by vitamin E as well.

Micronutrients in dengue:

There have only been a very limited number of experimental research conducted on dengue fever with vitamin supplementation. Modulation of nutritional status and the addition of micronutrients might be a cheaper and more cost-effective option. This fact has to be brought to your attention.

It was shown in a research carried out by Sánchez-Valdéz and colleagues in Mexico that treatment with calcium and vitamin D may enhance the results of dengue illness. [Citation needed] It was explained by the capacity of calcium to cause platelets to aggregate and by the ability of vitamin D to influence the production of IL-12 and the maturation of dendritic cells. [54]

According to the findings of a research carried out by Vaish et al[55], giving children who had dengue fever vitamin E supplementation improved their platelet count, but it did not have any effect on the severity of the condition. In their final comments, Ahmed et al.56 in their study noted the need of more research into the function that multivitamin supplements, including vitamin B and vitamin C, play in dengue.

Because of its antioxidant characteristics, vitamin C is particularly useful in the treatment of respiratory tract infections and pneumonia. These advantages have been shown again and again. It has been shown that vitamin C has a critical function in the metabolism of amino acids, hormones, and folate, in addition to playing a

significant role in raising the amount of iron that is absorbed by the stomach. Even though there have been no studies conducted in this area up to this point, there is reason to believe that the function that vitamin C plays in the creation of collagen and the maintenance of capillary integrity has promise in the treatment of dengue. [57,58]

Relevant studies:

In a study done by Vaish et al[59] in 2010 among 66 cases of dengue fever having thrombocytopenia. All patients were categorized into 2 groups and group II received standard management for dengue. It concluded that the MPC after 4days was significantly higher among patients of group I when compared with patients of group II (122.19 10 3 /min vs 92.57 10 3/min; P value 0.043) and the same findings was seen even after 7 days. Requirement for platelet was less among the patients of group Iwhen compared with group II cases.

In a study done by Chathurangana et al[60] among 127 cases in 2015 concluded that management with vitamin E showed significant improvement of clinical, hematological and bio-chemical parameters in children with dengue.

In a research that was carried out by Langerman SD et al[61], it was shown that extra vitamin C, D, E, and zinc may be beneficial adjuncts in the treatment of DF, and the purpose of the study was to shed light on the function that micronutrient supplementation plays in the treatment of DF.

In a study done by Ahmed S et al[62] in 2014 concluded that the role of micronutrients in dengue virus infection is an exciting research area and needs to be examined in well-designed studies with larger samples.

In a study done by Chandra P et al[63] in 2013 among 48 susepected dengue infection concluded that Vitamin E and C supplementation contribute to increase in platelet count and recovery in patients with dengue fever.

In a study done by Ramalingam K et al[64] in 2019 among 200 patients with dengue infection showed that the patients who received vitamin C supplementation had a greater percentage increase in platelet count and shorter hospital stay indicating significant association between vitamin C supplementation and duration of hospital stay. In a study done by Lakhanpal G et al[65] in 2016 among 110 cases of dengue infection showed that vitamin C may berecommended in all patients of dengue fever.

MATERIAL AND METHODS

STUDY SUBJECTS:

Children of age group between 5 years to 18 years diagnosed with dengue fever admitted in KLE'S DR. PRABHAKAR KORE HOSPITAL & RESEARCH CENTRE.

STUDY DESIGN:

Randomized Control Trail Study

STUDY PERIOD:

Data collection – 1 year (2021 January to 2021 December).

STUDY SETTING:

KLE Academy of Higher Education and Research, Dr. Prabhakar Kore Charitable Hospital, Department of Paediatrics, Jawaharlal Nehru Medical College, Belagavi.

INCLUSION CRITERIA:

1) Children between the ages of 5-18 years, & who fulfilled the clinical criteria of Dengue Fever (fever & 2 of the other symptoms: headache, retro-orbital pain, myalgia, arthralgia, bone pain & haemorrhagic manifestations) with diagnosed NS1AG Positive or dengue IgG & IgM Positive.
2) Patients of dengue fever (as per WHO criteria[6]) with thrombocytopenia (platelet count < 1.5lakhs).

EXCLUSION CRITERIA:

1) Patients of dengue fever with platelet counts > 1.5lakhs.
2) Patients who already started on Vitamin E & Vitamin C supplementation prior to admission.
3) Patients of dengue fever with severe thrombocytopenia requiring platelet transfusion.
4) Patients of dengue fever with severe GI bleeding manifestations.

SAMPLE SIZE:

Sample size is calculated by using **G*Power** software. Data will be collected at 8 different time points By assuming small effect size within the subject as 0.15, 5% level of significance, power 80%, non-sphericity correction ε taken as 1 and by assuming 0.5 correlation present between repeated measures, minimum sample size required is **42 per each group**.

Larger the sample, better the precision.

Tests will be used to analyse the data:

To check the normality of the variables Shapiro-Wilk's test/QQ plot will be used. To compare the mean/distribution of parameters within the time points (for more than two time points) One-way repeated measures of ANOVA/Friedman's test will be used. To check the dependency structure between two variables Chi-square test will be used. To compare mean/distributions at two different time points paired t-test/Wilcoxon's test will be used. To compare mean/distributions over two group's t-test/Mann-Whitney test will be used.

Table 2: Randomization table:

Randomization table obtained using statistical software **R** version 4.0.2.

GROUP	PATIENT NUMBER
CONTROL	5,7,9,10,11,13,14,15,19,21,23,26,29,30,32,33,34,36,40,43,45,47, 49,53,54,55,56,57,60,63, 64,65,67,68,69,71,76,78,79,81,83,84.
EXPERIMENTAL	1,2,3,4,6,8,12,16,17,18,20,22,24,25,27,28,31,35,37,38,39,41,42,44,46 ,48,50,51,52,58,59,61,62, 66,70,72,73,74,75,77,80,82.

Note:

Formula for sample size calculation is not required. Sample size was calculated based on two-way repeated measures of ANOVA model using **G*Power** software. **G*Power** software is specially designed for sample size calculation.

ETHICAL CONSIDERATION:

Institutional Ethical Committee approval, from Jawaharlal Nehru Medical College, Belagavi, was obtained before the start of the study. Informed written consent was obtained from the parents.

Source of Funding: None declared

Conflict of Interest: None declared

STUDY PROCEDURE:

Patients who fulfilled the above-mentioned criteria will be included in this study and randomly assigned using statistical software R version to either of 2 groups:

Group 1: Patients who receive Vit E (Tab. Evion 400MG) Once daily & Vit C (Tab. Immun 500MG) thrice a day for 5 days.

Group 2: Patients who receive standard treatment only.

Standard Treatment will be initiated in all patients after being randomly assigned to a group & will be continued for a period of 5 days.

Children who are not able to take orally & who prefer to have in liquid suspension, oral liquid will be given with the same strength of tablet formulation.

Oral liquid of Vit E: 26 IU/1ML

Oral liquid of Vit C: 100MG/1ML

After initiating Vit E & Vit C supplementation, the investigator will be confirming whether the patient is compliant with the treatment regimen.

The platelet count were measured daily for 5 days in all the groups.

We will study the effect of role of Vitamin E & Vitamin C supplements on thrombocytopenia in dengue fever.

BUDGET:

Self. (No added investigation or intervention)

STATISTICAL ANALYSIS:

Data entry was done using M.S. Excel and statistically analysed using Statistical package for social sciences (SPSS Version 16) for M.S Windows. Descriptive statistical analysis was carried out to explore the distribution of several categorical and quantitative variables. Categorical variables were summarized with n (%), while quantitative variables were summarized by mean ± S.D. All results were presented in tabular form and are also shown graphically using bar diagram or line diagram as appropriate. The difference in the two groups were tested for Statistical Significance using Parametric tests such as t-test. P-value less than 0.05 considered to be statistically significant.

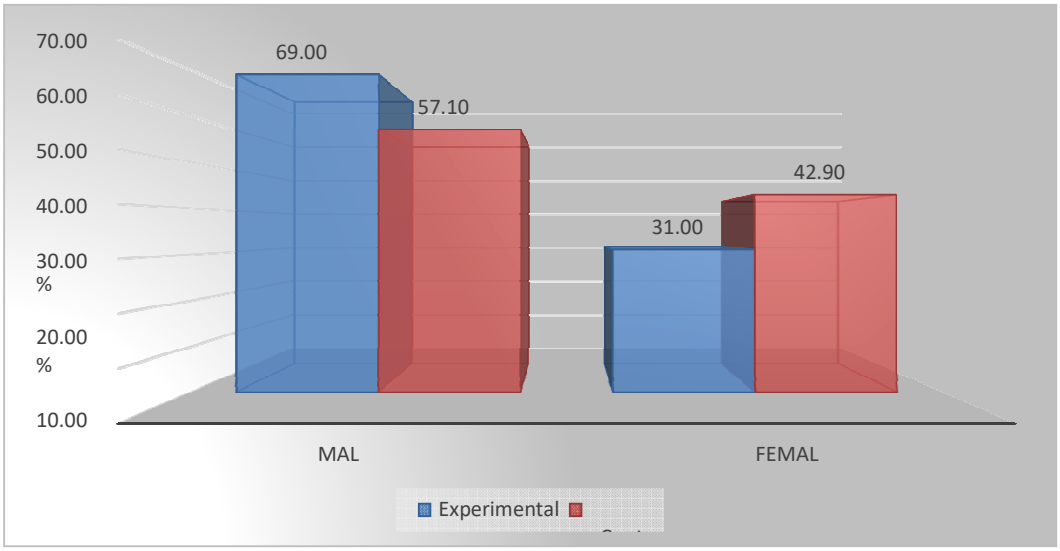
RESULTS

Table 3: Distribution of patients based upon the gender among experimental and controlgroups

			Group		Total
			Experimental	Control	
Gender	Male	n	29	24	53
		%	69.0%	57.1%	63.1%
	Female	n	13	18	31
		%	31.0%	42.9%	36.9%
Total		n	42	42	84
		%	100.0%	100.0%	100.0%

Chi-Square: 1.2, P Value: 0.18, Statistically not significant

Among experimental group, males were 69% and females were 31%. Among control group, males were 57.1% and females were 42.9%. There was no statistically significance found in between the groups.



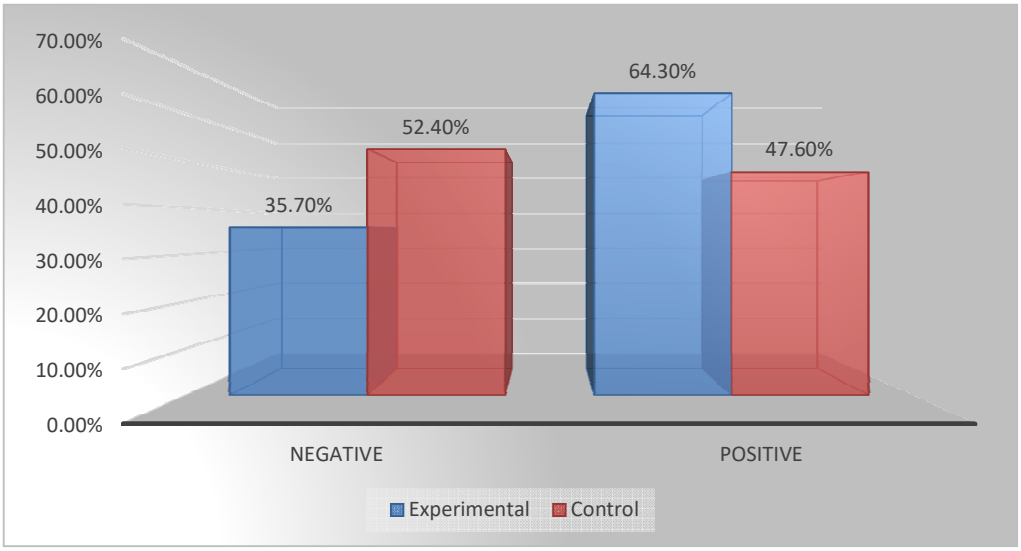
Graph 1: Distribution of patients based upon the gender among experimental and control groups

Table 4: Distribution of patients based upon the NS1 Ag among experimental andcontrol groups

			Group		Total
			Experimental	Control	
NS1 Ag	Negative	n	15	22	37
		%	35.7%	52.4%	44.0%
	Positive	n	27	20	47
		%	64.3%	47.6%	56.0%
Total		n	42	42	84
		%	100.0%	100.0%	100.0%

Chi-Square: 2.36, P Value: 0.09, Statistically not significant

In experimental group, NS1 Ag was positive in 64.3% patients; while in control group, NS1 Ag was positive in 47.6%. The association between the groups was foundto be statistically not significant.



Graph 2: Distribution of patients based upon the NS1 Ag among experimental and control groups

Table 5: Distribution of patients based upon the IgG/IgM among experimental and control groups

			Group		Total
			Experimental	Control	
	Equivocal	n	0	4	4
		%	0.0%	9.5%	4.8%
	IgG - IgM +	n	1	0	1
		%	2.4%	0.0%	1.2%
	IgG + IgM-	n	0	1	1
		%	0.0%	2.4%	1.2%
	IgG +ve	n	9	8	17
		%	21.4%	19.0%	20.2%
	IgM +ve	n	6	7	13
		%	14.3%	16.7%	15.5%
	Negative	n	2	1	3
		%	4.8%	2.4%	3.6%
	Positive	n	24	21	45
		%	57.1%	50.0%	53.6%
Total		n	42	42	84
		%	100.0%	100.0%	100.0%

Chi-Square: 6.66, P Value: 0.35, Statistically not significant

In experimental group, IgG/IgM was positive in 57.1% patients, IgG - IgM + in 2.4% patients, IgG positive in 21.4% patients and IgM positive in 14.3% patients.

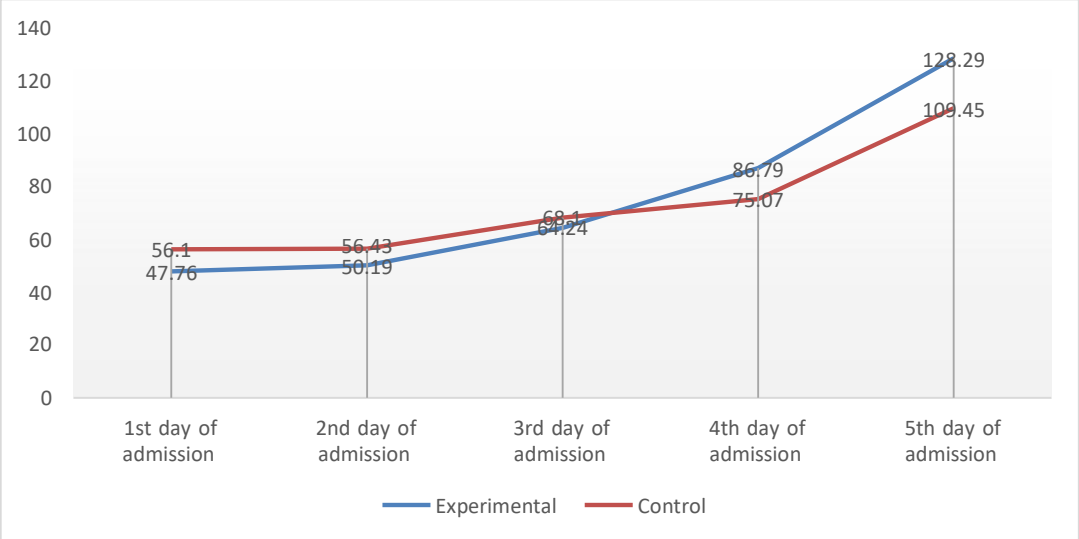
In control group, IgG/M was positive in 50% patients, IgG + IgM- in 2.4% patients, IgG positive in 90% patients and IgM positive in 16.7% patients. There was no statistically significance found in between the groups

Table 6: Comparison of platelet count between experimental and control groups

	Experimental		Control		T Test	P Value
	Mean	SD	Mean	SD		
1st day platelets	47.76	38.899	56.10	55.124	-0.80	0.42
2nd day platelets	50.19	32.323	56.43	50.343	-0.67	0.50
3rd day platelets	64.24	35.448	68.10	48.941	-0.41	0.68
4th day platelets	86.79	40.480	75.07	47.036	1.22	0.22
5th day platelets	128.29	55.300	109.45	85.764	1.19	0.23

In experimental group, mean platelet count on first day of admission was 47.76 and on 5th day of admission mean platelet count was 128.29 thousand cells/uL. There is 269% increase in platelet count from first day to 5th day of admission.

In Control group, first day mean platelet count on first day of admission was 56.10 and on 5th day of admission mean platelet count was 109.45 thousand cells/uL. There is 195% increase in platelet count from first day to 5th day of admission.



Graph 3: Comparison of platelet count between experimental and control groups

Table 7: Correlation of platelet count from 1st day of admission to 5th day of admissionamong experimental and control groups

Group		Correlation Coeff	P Value
Experimental	1st day platelets & 2nd day platelets	0.827	0.001
	1st day platelets & 3rd day platelets	0.560	0.001
	1st day platelets & 4th day platelets	0.318	0.040
	1st day platelets & 5th day platelets	0.102	0.520
Control	1st day platelets & 2nd day platelets	0.939	0.001
	1st day platelets & 3rd day platelets	0.663	0.001
	1st day platelets & 4th day platelets	0.568	0.001
	1st day platelets & 5th day platelets	0.128	0.418

In both experimental and control group, there is statistically significant correlation fromfirst day of admission to 4th day.

DISCUSSION

This study was conducted at the Department of Paediatrics, KLE Dr. Prabhakar Kore Hospital and Medical Research Centre. Total of 84 patients were included in the study. 42 patients were included in each group. Patients who receive Vit E (Tab. Evion 400MG) Once daily & Vit C (Tab. Immun 500MG) thrice a day for 5 days comprise group 1. Patients who receive standard treatment only comprise group 2. Children between the ages of 5-18 years, & who fulfilled the clinical criteria of Dengue Fever (fever & 2 of the other symptoms: headache, retro-orbital pain, myalgia, arthralgia, bone pain & haemorrhagic manifestations) with diagnosed NS1AG Positive or dengue IgG & IgM Positive were included. The present study was initiated to evaluate the effect of vitamin e & vitamin c supplementation on thrombocytopenia in children between 5yrs to 18yrs with dengue fever.

GENDER

In the present study, among experimental group, males were 69% and females were 31%. Among control group, males were 57.1% and females were 42.9%. There was no statistically significance found in between the groups. In Ramalingam K et al.,[64] Majority of Male patients (58.5%) were found to be affected with dengue fever compared to Female patients (41.5%).

NS1 Ag

In the present study, In experimental group, NS1 Ag was positive in 64.3% patients; while in control group, NS1 Ag was positive in 47.6%. The association between the groups was found to be statistically not significant.

IG G/M

IgM and IgG have been extensively used for diagnosis of DHF. IgM antibodies, first to appear are detectable in 50% of patients by days 3-5 after onset of illness, increasing to 80% by day 5 and 99% by day 10. IgM levels peak about two weeks after the onset of symptoms and then decline generally to undetectable levels over 2-3 months. Anti-dengue serum IgG is generally detectable at low titers at the end of thefirst week of illness, increasing slowly thereafter, with serum IgG still detectable afterseveral months, and probably even for life. During a secondary dengue infection (a dengue infection in a host that has previously been infected by a dengue virus, or sometimes after non-dengue flavivirus vaccination or infection), antibody titres rise rapidly and react broadly against many flavi viruses. The dominant immunoglobulin is IgG which is detectable at high levels, even in the acute phase, and persists for periods lasting from 10 months to life. In, early convalescent stage IgM levels are significantly lower in secondary infections and may be undetectable in few, depending on the test used.[68]

In the present study, In experimental group, IgG/M was positive in 57.1% patients, IgG - IgM + in 2.4% patients, IgG positive in 21.4% patients and IgM positive in 14.3% patients. In control group, IgG/M was positive in 50% patients, IgG + IgM- in 2.4% patients, IgG positive in 90% patients and IgM positive in 16.7% patients. There was no statistically significant difference between the groups.

PLATELET COUNT

In the present study, In experimental group, mean platelet count on first day of admission was 47.76 and on 5th day of admission mean platelet count was

128.29 thousand cells/uL. There is 269% increase in platelet count from first day to 5th day of admission. In Control group, first day mean platelet count on firstday of admission was 56.10 and on 5th day of admission mean platelet count was 109.45 thousand cells/uL. There is 195% increase in platelet count from first day to 5th day of admission.

In Chathurangana PW et al.,[5] mean platelet count of the vitamin E group remained higher than the placebo group from Day 3 to Day 7 of the illness. It has

shown that vitamin E treatment significantly improves these parameters in children with DF and DHF. Platelet counts were also higher in the vitamin E group although this did not reach statistically significant levels.

In Ramalingam K et al.,[64] average percentage increase in platelet count was greater (363.12%) among patients administered with vitamin C than those who were not given vitamin C (105.70%), and a *p-value < 0.0001 indicates that the results were highly significant.

In Vaish et al.[7] the patients were randomized to two groups – group I which received vitamin E 400 mg (Evion, Merck) once daily along with standard treatment and group II which received standard treatment only. They focused on the platelet count of dengue patients who received vitamin E treatment during the illness. They demonstrated higher platelet counts in patients who were on vitamin

E. On day 1, the mean platelet counts were higher but there was no significant difference between the two groups (P = 0.3545). On day 4 and 7, the mean platelet counts in group I cases were significantly higher than the mean platelet counts on

these days in group II. These Findings indicate that with passage of time, the platelet counts increased in both the groups, the increase was faster and greater in the group I receiving vitamin E 400 mg/daily with significant differences being apparent on day 4 and day 7.

These findings suggest that vitamin E treatment reduces the bone marrow effectsof the dengue virus and also helps in faster recovery.

Vitamin E supplementation was associated with improvements in various clinical and hematological parameters. [5]

Vitamin E can fasten the increase of the circulation with platelets in dengue fever, thereby reducing the risk of bleeding, particularly during the initial period when the risk is greatest. As vitamin E is simple, safe to administer, this would be a valuable effect and there would be no harm in using it in such patients.

Vitamin C might fasten the increase the platelets in dengue fever, thereby reducing the need for platelet

transfusion. As Vitamin C is available at low cost and is relatively free of serious adverse effects, it may be recommended to all patients of Dengue fever.

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 iftreatment is started on day one itself there may be more significant benefits to patients. Based on our findings, we recommend that vitamin E and vitamin C should be incorporated in the routine management of DF/DHF in children. This will help in determining the effects of vitamin E and vitamin C treatment on overall disease outcomeand in optimising the dose of the vitamin E and vitamin C. A larger sample would give an insight into the overall disease outcome in children.

STRENGTH

There are only a few studies, especially in India. This study will help to evaluate the effect of vitamin e & vitamin c supplementation on thrombocytopenia in children between 5yrs to 18yrs with dengue fever.

The study was conducted during the period of the Covid-19 restrictions and despite these challenges during the study period in the collection of data, the desired sample size was collected.

The data was collected primarily by the principal investigator, and hence the information bias and interobserver bias will be minimal.

SUMMARY AND CONCLUSION

Background and objectives:

Dengue virus infection is the most widespread mosquito-borne viral infection in humans and has emerged as a serious global health challenge. The major threat of dengue fever is from thrombocytopenia and consequently from the bleeding manifestations. The pathogenesis of thrombocytopenia in dengue fever is not clear but increased oxidative stress and micronutrients supplementation may have a role. Vitamin E which has antioxidant and Vitamin C stimulate immune system function may help in these cases. The present was conductedto evaluate the effect of vitamin E & vitamin C supplementation on thrombocytopenia in children between 5yrs to 18yrs with dengue fever.

Methods:

A randomized controlled trial was conducted at KLE Dr. Prabhakar Kore Hospital and Medical Research Centre for one year. Children between the ages of 5-18 years, & who fulfilled the clinical criteria of Dengue Fever (fever & 2 of the other symptoms: headache, retro-orbital pain, myalgia, arthralgia, bone pain & haemorrhagic manifestations) with diagnosed NS1AG Positive or dengue IgG & IgM Positive were included. 42 patients were included in each group. **Group 1:** Patients who receive Vit E(Tab. Evion 400MG) Once daily & Vit C (Tab. Immun 500MG) thrice a day for 5 days.**Group 2:** Patients who receive standard treatment only. After initiating Vit E & Vit C supplementation, the investigator confirms whether the patient is compliant with thetreatment regimen. The platelet count was measured daily for 5 days in all the groups. (c) Results: Vitamin C and vitamin E supplementation was given for five days in all the patients in experimental group. In experimental group, there is 269% increase in platelet count from first day to 5th day

of admission. In Control group, there is 195% increase in platelet count from first day to 5th day of admission. In both experimental and control group, there is statistically significant correlation from first day of admission to 4^{th} day.

Conclusion:

Vitamin E & vitamin C have shown beneficial effect on thrombocytopenia in dengue fever and results in faster increase in the platelet counts.

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ANNEXURE I – CONSENT FORM

INFORMED CONSENT FORM

Title of the study: "EFFECT OF VITAMIN E & VITAMIN C SUPPLEMENTATION ON THROMBOCYTOPENIA IN DENGUE FEVER" – A RANDOMISED CONTROLLED TRIAL

Respected Sir/Madam,

We invite you to participate in our study as you are eligible for the same. During the study, you will be asked some questions in detail regarding your present complaints.

Purpose of the study:

To evaluate the effect of Vitamin E & Vitamin C supplementation on thrombocytopenia in children between 5yrs to 18yrs with dengue fever.

Risks and Benefits:

The result of you taking part in this research would help health care providers towards a better understanding adverse effect of the immunisation process, and thus we will be able to provide improved patient care.

Alternatives:

If you decide not to participate in this study, you will still be receiving the usual standard of care.

Privacy and confidentiality:

Your privacy will be respected, and all information collected about you during this study will be kept confidential. Your identity will remain undisclosed.

Relations with the Institutional policy:

The J N Medical College will provide, within the limitations of the laws of the State of Karnataka, facilities and medical attention to patients who suffer injuries because of participating in this project.

Financial incentives:

You shall not be receiving any payment or any financial incentives for participating in this study.

Authorization to publish results:

The results of this study may be published for scientific purpose or presented to a scientific group. Your identity, however, will always be maintained confidential.

Voluntary participation:

Your participation in this study is voluntary. Your decision whether to participate will neither affect the care of your current disease, nor your future relations with the doctor or the hospital. In the event, if you suffer any physical injury as the result of your participation in this study, you may contact the investigator of the study.

In the event of an emergency, you should contact KLE'S Dr. Prabhakar Kore Hospital and MRC on Telephone No. 08312473777.

In case you need further information regarding your rights as a study participant, you may please contact DR HARSHA HEGDE, CHAIRPERSON, KLE ACADEMY OF HIGHER EDUCATION & RESEARCH,

JAWAHARLAL NEHRU MEDICAL COLLEGE,

IEC AND SCIENTIST D, ICMR

BELAGAVI- 590010,

9480422500

STATEMENT OF CONSENT

I, Mr./Ms/Mrs. _____ Parent/Guardian volunteer of _____ consent to participate in this study. I have read the consent document, or it has been read to me in my vernacular language. I accept to participate in the study. All the information regarding this study is provided to me and I have understood the same. I have been given the opportunity to ask questions and obtain appropriate answers.

The signature or left thumbprint of participants Parent/Guardian:

Signature of the investigator:

Date:

Participant Informed Consent Form: Assent (<18years)

Protocol /Study No.:

Patient Id No. for this trial:

Project Title: "EFFECT OF VITAMIN E & VITAMIN C SUPPLEMENTATION ON THROMBOCYTOPENIA IN DENGUE FEVER" – A RANDOMISED CONTROLLED TRIAL

Name of Principal Investigator: Dr. BHUVANAGIRI SRIKANTH

Tel. No(s): 8686144466

Contact address: Junior Resident, Department of Paediatrics, JNMC, KLE BELAGAVI

The content of information sheet dated that was provided have been read carefully by me/explained to me in detail to me, in a language that I comprehend and have fully understood its content. I confirm that I have had opportunity to ask questions.
The nature and purpose of study and its potential risks/benefits and expected duration of study, and relevant details of study have been explained to me in detail. I understand that my participation is voluntary and that I am free to withdraw at any time without giving reasons, without my medical care or legal rights being affected.
I understand that information collected about me from my participation in this research and section of any of my medical notes, may be looked at by responsible individuals from regulatory authorities where relevant to taking part in research. I give permission for these individuals to have access to my record

Signature/left thumb impression
Signature of the subject/left thumb impression & Date: _____
Place: _____
Name of the participant: _____
Name of the parent/LAR: _____
Son/daughter/spouse of: _____

This is to certify that above consent has been obtained in my presence.

Name of Principal Investigator **Signature of Principal Investigator**

Name of Witness 1
Signature of Witness 1
Address:

Name of Witness 2
Signature of Witness 2
Address:

PROFORMA

"EFFECT OF VITAMIN E & VITAMIN C SUPPLEMENTATION ON THROMBOCYTOPENIA IN DENGUE FEVER" – A RANDOMISED CONTROLLED TRIAL

Overall Serial No:

IP Number:

Date:

Socio-demographic Particulars:

Informant's Name:

Relation:

Profession:

Education:

Permanent Residence:

Contact Telephone:

Patient's Name:

Gender: Male/Female

Date of Admission:

Age:

NS1AG:

IgG:

IgM:

VITAMIN E:

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
VITAMIN E					

VITAMIN C:

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
VITAMIN C					

PLATELET COUNT:

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Platelet Count					
PCV					

(Signature of Investigator)

sno	IP No	Sex	control/experiment	Vit C	Vit E	NS1 Ag	IG G/M	1st day plts	2nd day plts	3rd day plts	4th day plts	5th day plts
1	1028511	m	experimental	5 days	5 days	positive	IgG +ve	56K	88K	92K	109K	121K
2	1028781	m	experiment	5days	5 days	positive	positive	18	33	67	121	184
3	1029315	m	experiment	5days	5days	positive	positive	30	12	33	70	135
4	1029579	m	experiment	5 days	5days	positive	positive	31	29	39	12	71
5	1031143	m	control	not taken	not taken	negative	positive	224	211	198	212	178
6	1031744	m	experiment	5 days	5 days	positive	positive	15	8	42	31	49
7	1033587	m	control	not taken	not taken	positive	positive	26	19	57	71	75
8	1034572	m	experiment	5 days	5 days	positive	IgG +ve	34	36	38	101	224
9	1034923	f	control	not taken	not taken	positive	positive	32	34	48	61	556
10	1033356	m	control	not taken	not taken	positive	positive	17K	35K	56K	66K	72K
11	1039271	m	control	not taken	not taken	positive	positive	50	45	64	84	197
12	1037728	m	experiment	5 days	5 days	positive	negative	13	47	127	128	271
13	1042716	m	control	not taken	not taken	negative	equivocal	12	49	171	172	173
14	1043398	m	control	not taken	not taken	negative	igg +igm-	12	49	171	172	223
15	1043398	f	control	not taken	not taken	negative	equivocal	33	33	43	56	119
16	1044575	m	experiment	5 days	5 days	positive	positive	14	22	21	34	43
17	1048425	m	experiment	5 days	5 days	positive	positive	137	112	94	90	126
18	1045416	m	experiment	5 days	5 days	positive	positive	24	52	46	64	66
19	1045859	m	control	not taken	not taken	positive	positive	28	14	17	36	95
20	1040543	f	experiment	5 days	5 days	positive	positive	20	29	32	60	100
21	1046781	m	control	not taken	not taken	positive	negative	24	30	32	45	90
22	1047740	f	experiment	5 days	5 days	positive	igg -igm+	74	70	75	61	102
23	1043398	f	control	not taken	not taken	negative	positive	33	43	43	56	75
24	1044575	m	experiment	5 days	5 days	positive	positive	9	9	22	22	34
25	1045589	m	experiment	5 days	5 days	positive	positive	28	16	14	14	17
26	1042716	m	experiment	5 days	5 days	positive	positive	12	48	98	177	223
27	1046781	m	experiment	5 days	5 days	positive	positive	50	28	53	64	112
28	1047740	f	control	not taken	not taken	positive	positive	124	68	78	85	96
29	1050464	m	control	not taken	not taken	positive	positive	90	129	129	180	134
30	1050460	f	control	not taken	not taken	positive	positive	70	80	112	120	150
31	1050462	f	experimental	5 days	5 days	positive	IgM +ve	70K	80K	112K	120K	150K
32	1051278	f	control	not taken	not taken	positive	IgM +ve	36K	32K	34K	56K	78K
33	1052410	f	control	not taken	not taken	positive	positive	14K	24K	56K	78K	128K
34	1054111	f	control	not taken	not taken	negative	IgM +ve	128K	118K	103K	89K	126K
35	1054381	m	experimental	5 days	5 days	negative	positive	131K	104K	142K	153K	156K
36	1055048	m	control	not taken	not taken	negative	IgG +ve	12K	21K	35K	54K	72K
37	1055084	f	experimental	5 days	5 days	negative	positive	14K	38K	45K	55K	123K
38	1055202	m	experimental	5 days	5 days	negative	positive	22K	14K	83K	130K	162K
39	1056076	f	experimental	5 days	5 days	positive	positive	53K	41K	34K	53K	85K
40	1056711	m	control	not taken	not taken	negative	positive	14K	17K	30K	42K	60K
41	1056690	m	experimental	5 days	5 days	negative	IgG +ve	46K	87K	115K	120K	122K
42	1056989	f	experimental	5 days	5 days	positive	IgG +ve	17K	13K	23K	73K	200K
43	1057347	m	control	not taken	not taken	negative	IgG +ve	48K	45K	39K	23K	160K
44	1057349	f	experimental	5 days	5 days	negative	positive	123K	61K	46K	60K	102K
45	1057695	f	control	not taken	not taken	negative	IgM +ve	77K	38K	31K	32K	27K
46	1057966	f	experimental		5days	positive	positive	34k	40k	56k	80k	117k
47	1058332	m	control	not taken	not taken	negative	positive	210k	207k	161k	147k	145k
48	1058737	m	experimental	5 days	5 days	positive	IgM +ve	161K	111K	85K	105K	118K
49	1059293	f	control	not taken	not taken	negative	IgM +ve	197K	196K	177K	152K	134K
50	1059517	m	experimental	5 days	5 days	negative	IgG+ve	60k	62k	67k	105k	140k
51	1060187	m	experimental	5 days	5 days	negative	positive	33k	21k	32k	72k	99k
52	1060817	m	experimental	5 days	5 days	negative	IgM +ve	11K	15K	40K	69K	80K
53	1060568	f	control	not taken	not taken	positive	IgG+ve	31K	56K	87K	23K	44K
54	1061159	m	control	not taken	not taken	positive	positive	89k	92k	98k	103k	157k
55	1061229	m	control	not taken	not taken	negative	IgG+ve	41k	23k	20k	26k	38k
56	1061940	f	contol	not taken	not taken	negative	IgG+ve	29k	33k	44k	49k	83k
57	1062200	f	control	not taken	not taken	negative	IgM +ve	33K	21K	16K	18K	42K
58	1062265	f	experimental	5 days	5 days	negative	IgG+ve	28K	42K	47K	95K	139K
59	1062437	f	experimental	5 days	5 days	negative	IgG+ve	16K	45K	60K	139K	160K
60	1065326	m	control	not taken	not taken	negative	positive	136k	95k	79k	68k	80k
61	1065813	m	experimental	5 days	5 days	positive	IgM +ve	19K	17K	18K	30K	43K
62	1066026	M	experimental	5 days	5 days	negative	IgM +ve	44K	38K	35K	70K	140K
63	1066836	F	control	not taken	not taken	negative	IgM +ve	21K	31K	45K	79K	160K
64	1067269	M	control	not taken	not taken	positive	positive	33K	26K	32K	40K	46K
65	1067282	M	control	not taken	not taken	positive	positive	10K	12K	14K	40K	39K
66	1067699	M	experimental	5 days	5 days	negative	IgG +ve	16K	39K	44K	88K	143K
67	1067581	M	control	not taken	not taken	negative	IgG +ve	10K	20K	14K	47K	51K
68	1069443	M	control	not taken	not taken	positive	positive	30K	25K	28K	70K	74K
69	1069722	M	control	not taken	not taken	negative	positive	75K	63K	92K	102K	138K
70	1071681	F	experimental	5 days	5 days	positive	negative	64K	45K	61K	75K	170K
71	1072123	M	control	not taken	not taken	positive	IgM +ve	120k	99k	77k	72k	76k
72	1072507	m	experimental	5 days	5 days	negative	positive	73K	58K	81K	92K	142K
73	1073081	M	experimental	5 days	5 days	negative	positive	53K	71K	99K	112K	140K
74	1073975	M	experimental	5 days	5 days	positive	IgM +ve	120K	132K	152K	145K	180K
75	1074551	M	experimental	5 days	5 days	negative	IgG +VE	51K	73K	87K	140K	230K
76	1075704	f	control	not taken	not taken	positive	equivocal	26K	30K	52K	45K	68K
77	1076249	M	experimental	5 days	5 days	positive	positive	15K	28K	37K	52K	96K
78	1076551	m	control	not taken	not taken	positive	equivocal	22K	16K	33K	20K	21K
79	1076704	f	control	not taken	not taken	negative	positive	14K	29K	33K	68	77K
80	1077482	f	experimental	5 days	5 days	positive	positive	90K	104K	117K	152K	161K
81	1077964	f	control	not taken	not taken	negative	IgG +ve	54K	66K	89K	74K	90K
82	1078149	f	experimental	5 days	5 days	positive	positive	77K	90K	87K	102K	112K
83	1078317	f	control	not taken	not taken	positive	positive	42K	66K	78K	74K	80K
84	1078590	m	control	not taken	not taken	negative	IgG +ve	29K	30K	44K	46K	70K