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Research Article

ROLE OF ANTIOXIDANT VITAMIN E AND C ON PLATELET LEVELS IN DENGUE FEVER

***Prasanna Chandra[1], Hemant Sharma[2], Akash Gupta1[1] and Yogesh Rai[1]**
[1]*Department of Biochemistry, SIMS, Hapur, U.P.*
[2]*Department of Medicine, SIMS, Hapur, U.P.*

ABSTRACT
The aim of the study was to assess the beneficial effects of vitamin E and C on thrombocytopenia in dengue patients. Blood samples of 48 suspected cases of dengue from medicine ward were collected. Serum antibodies (IgG, IgM) were detected by ELISA based readymade kits from J Mitra and company. Hemogram and complete blood count was also done. Only 27 fully diagnosed cases of dengue were given vitamin E and C supplementation according to prescribed limit for 8 days. Hb and platelet count was estimated on day 1 and Day 8. Data was analyzed by paired t test. In our study we found that mean Hb value was 10.2 ± 0.4 gm/dl on day 1 and it was 10.9 ± 0.3 gm/dl on day 8. Similarly mean platelet count was $1.8 \pm 0.3 \times 10^5$ on day 1 and $2.3 \pm 0.5 \times 10^5$ on day 8 that is after supplementation. Vitamin E and C supplementation may contribute to increase in platelet count and early recovery in dengue fever.

Key Words: *Dengue fever, Dengue Hemorrhagic Fever, Thrombocytopenia, Vitamin E, Vitamin C.*

INTRODUCTION
The word dengue is derived from the Swahili phrase Ka-dingo pepo meaning cramp like seizure. Dengue viruses (DV) belong to family Flaviviridae and there are four serotypes of the virus reffered to as DV-1, DV-2, DV-3 and DV-4. It is transmitted mainly by *Aedes aegypti* mosquito and also by *Aedes albopictus*. In India, the A*edes aegypti* mosquitoes are primary vectors for DENV and CHIKV which are more prevalent in the low socio-economic conditions and high population density (Scott *et al.,* 1997). All four serotypes can cause the full spectrum of disease from a subclinical infection to a mild self limiting disease, the Dengue fever (DF) and a severe disease that may be fatal, the dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) (Nivedita *et al.,* 2012). The first major wide spread epidemics DHS/DSS occurred in India in 1996 involving areas around Delhi and Lucknow and then it spread to all over the country (Dar *et al.,* 1999; Agarwal *et al.,* 1999; Shah *et al.,* 2008). In Delhi till 2003 the predominant serotype was DV-2 (genotype IV) but in 2003 for the first time all four Dengue virus subtypes were found to co-circulate in Delhi thus changing it to a hyperendemic state followed by complete predominance of DV serotype 3 in 2005 (Dar *et al.,* 1999; Gupta *et al.,* 2006). The classical clinical presentation of Dengue virus infection includes fever, joint pains, headaches and rashes (petechial or maculopaular rash) usually involving the limbs and trunks are the characteristic symptoms of the disease. In addition, thrombocytopenia is more marked and develops earlier in cases with a fatal outcome (Fausi *et al.,* 2008). However many other types of clinical presentations have been observed.They are neurological, hepatic, cutaneous and gastrointestinal. Among neurological manifestation encephalopathy, acute motor weakness, seizures, neuritis, hypokalemic paralysis are common (Misra *et al.,* 2006; Verma *et al.,* 2012; Kumar *et al.,* 2008; Verma *et al.,* 2011). In the hepatic involvement acute liver failure, hepatic encephalopathy, hepatomegaly and jaundice are commonly seen (Deepak *et al.,* 2006; Vinodh *et al.,* 2005; Giri *et al.,* 2008; Jhamb *et al.,* 2011). In the gastrointestinal (GI) system lower GI bleeding and acute inflammatory colitis are seen (Rama *et al.,* 2006). Hemophagolytic syndrome includes bone marrow heamophagocytosis associcated with nasal bleeding and pancytopenia (Jain *et al.,* 2008; Ray *et al.,* 2011; Kapdi *et al.,* 2012). Cytotoxic factor (CF), a unique cytokine, is produced by CD4+ T cells in DV infected mice and man. Most of the patients with Dengue virus infection have CF in their sera, with peak amounts in the most severe cases of DHF (Mukarjee *et al.,* 1997; Chaturvedi *et al.,* 1999). CF appears in the serum before the clinical illness and is present in 100 percent patients with DF/DHF up to day 4 of illness,

detectable up to 20 days of illness (Chadurvedi *et al.*, 2006). Macrophages are the primary component of the host innate immune system and provide first line of defence against viral infections. But in Dengue viral infection, the macrophages play multiple paradoxical roles, sometimes these help in eradicating the virus, while sometimes these actually increase its replication within the host (Chaturvedi *et al.*, 2006). CF-2 is a biological active protein and causes various immunopathological effects including increased vascular permeability and damage to the blood brain barrier (Khanna *et al.*, 1990; Dhawan et al. 1990; Chaturvedi *et al.*, 1986; Chaturvedi *et al.*, 1989). DV-2 inhibits in vitro megakaryopoiesis and induces apoptotic cell death in subpopulation of early megakaryocytic progenitors which may contribute to thrombocytopenia in dengue disease (Basu *et al.*, 2008). In another study it was shown that DV-2 may directly interact with and activate platelets and thus may be responsible for thrombocytopenia (Ghosh *et al.*, 2008). Oxidative stress develops from the onset of Dengue infection. Increase in oxidative stress significantly elevates plasma protein carbonyl and lower the protein bound sulfhydryl group. This ratio is reported to cause DHF/DSS (Soundravally *et al.*, 2008; Soundravally *et al.*, 2008).

MATERIALS AND METHODS

The study was conducted in Saraswathi Institute of Medical Sciences in the Department of Medicine in collaboration with Biochemistry, after obtaining ethical clearance from the institutional ethical committee. Patients were recruited for the study after taking their due written consent. Blood samples of 48 suspected Dengue cases from medicine wards were collected during a period from October 2012 to December 2012. Sera were separated for antidengue IgM and IgG antibody, which were tested by ELISA kits from J Mitra and Co in the Microbiology Department. Hemogram including platelet count and complete blood count was done in Pathology department.
Out of 48 patients only 27 were included in our study (17 males and 10 females) and all of these were having platelet count within normal reference range ($1.5 – 4 \times 10^5$ / cu mm). These 27 patients were given Vitamin E and C in the prescribed limit. 400 mg of Vit E (Cap Evion) was given once daily for 8 days and 500mg of Vit C (Tab Celin) was given thrice a day for 8 days. After initiating Vit E and Vit C supplementation there platelet count were measured first on Day 1 and again on Day 8. The remaining 21 patient were excluded from the study. The exclusion criteria were h/o bleeding disorder, severe thrombocytopenia requiring platelet transfusion, severe anemia and seriously ill patients. Finally Data was analyzed by paired t test.

RESULTS AND DISCUSSION

Present study involves the role of Vit E and C on platelets in Dengue Fever. In our study we found that mean Hb value was 10.2 ± 0.4 gm/dl at Day 1 and it was 10.9 ± 0.3 gm/dl at Day 8. Similarly mean platelet count was $1.8 \pm 0.3 \times 10^5$ at Day 1 and $2.3 \pm 0.5 \times 10^5$ at Day 8. It was found that mean platelet count was increased significantly by Vit E and Vit C supplementation (*P* value < 0.01) in Dengue patients (Table 1):

	Day 1	**Day 8**	***P* value**
Mean Hb	10.2 ± 0. 4 gm/dl	10.9 ± 0.3 gm/dl	$P < 0.01$
Mean platelet count	$1.8 \pm 0.3 \times 10^5$/ cu mm	$2.3 \pm 0.5 \times 10^5$/ cu mm	$P < 0.01$

Dengue infection is no more an urban area infection but it has penetrated in rural areas also (Nivedita *et al.*, 2012). The management of Dengue virus infection is essentially supportive and symptomatic. No specific treatment is available. However, there are Indian studies which have contributed in terms of better management of DHF/DSS. The pathogenesis of thrombocytopenia in Dengue fever is not clear but increased oxidative stress may have a role (Gil *et al.*, 2004; Klassen *et al.*, 2004). A rapid response to platelet and fresh frozen plasma transfusion is reported a study (Choudhry *et al.*, 2006). The role of

platelet transfusion in the management of hospitalized Dengue patients may vary for indication and situations. Vitamin E which has antioxidant and free radical scavenging properties, may therefore help in these cases (Sies *et al.,* 1995; Mascio *et al.,* 1991). Similarly Vaish et al observed that supplementation of antioxidant Vitamin E increased the count in dengue patients and they also concluded that Vitamin E can accelerate replenishment of the circulation with platelets in dengue fever, thereby reducing the risk of bleeding (Arvind *et al.,* 2012).

Vitamin E and C stimulate immune system function in supplementation studies (Hennekens, 1994). CF/CF2 induces macrophages to produce free radicals, nitrite, reactive oxygen and peroxynitrite (Misra *et al.,* 1996; Misra *et al.,* 1996; Chaturvedi *et al.,* 2006; Chaturvedi *et al.,* 2000; Misra *et al.,* 1998; Chaturvedi *et al.,* 1997).The free radicals, beside killing the target cells by apoptosis also directly up regulate production of pro-inflammatory cytokines; interleukin (IL-1), tumor necrosis factor (TNF-α), IL-8 and hydrogen peroxide in macrophage (Nivedita *et al.,* 2012). Bleeding manifestation is highly variable and do not always correlate with the laboratory abnormalities in the coagulation profile. Factors like mild degree of disseminated intravascular coagulation (DIC), hepatic derangement and thrombocytopenia act synergistically to cause bleeding in dengue patient (Shivbalan *et al.,* 2004). Severe bleeding is related to severe thrombocytopenia (Chairulfatah *et al.,* 2003). Reduced levels of plasma ascorbate and tocopherol may contribute to oxidative destruction of thrombocytes. So from our study we come to conlusion that administration of antioxidant Vitamin E and C along with the treatment may be fruitful to avoid thrombocytopenia in dengue patients.

Conclusion

Vitamin E and C supplementation may contribute to increase in platelet count and early recovery in dengue fever.

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