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Current knowledge of dengue pathogenesis and potential role of *Carica papaya* and vitamins in dengue fever

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Abstract. A number of biological molecules such as inflammatory enzymes and cytokines are altered during dengue virus (DENV) infection, many of which are attributed to the pathogenesis of the DENV infection. Papaya (*Carica papaya*) based extracts (PBE) and certain vitamins have been proven beneficial for dengue fever (DF) patients. The extract of papaya leaves were shown to improve platelet count in dengue patients. Extracts of papaya pulp based was also shown to induce *in vitro* IL-6 and stem cell factor in human peripheral blood mononuclear cells and stem cells of mesenchymal origin. Different vitamins such as D, E, and C have shown promise to treat dengue disease when taken in small supplementation trials. However, the exact molecular mechanisms on the beneficial roles of either PBE or vitamins are yet to be defined. Hence, the aim of this review is to link the cellular and molecular responses of DENV pathogenesis and pharmacological actions of the bio-active components of PBE or vitamins. It has also been shown that the beneficial roles of PBE and vitamins in DF are linked to thrombopoiesis, prevention of the viral entry and replication, decrease in oxidative damage assisted thrombocytopenia, and the reduction in vascular leakage. The DENV mediated fatalities are expected to expand it's geographic boundary whilst an efficient drug and the most likely candidate vaccine against DENV are still in progress. Findings on the molecular mechanisms of food and nutrient supplement might reinforce ongoing research to treat the increasing number of DENV infected patients using natural products while waiting for the right drug and vaccine.

INTRODUCTION

There is a spectrum of diseases related to dengue virus (DENV) infection that are commonly known as dengue fever (DF) (WHO, 1997). With the addition of symptoms and severity, the disease is categorized as dengue hemorrhagic fever (DHF). Patients with DHF may develop dengue shock syndrome (DSS) which results in plasma leakage and can be fatal.

In the year 2010, about 96 million people were infected globally by DENV, and in Asian countries alone, the number was 67 million (Bhatt *et al.*, 2013). Approximately 50-200 million cases of DENV infections, 500,000 episodes of either DHF or DSS, and over 20,000 dengue related deaths occur annually (Murray *et al.*, 2013). According to another study, almost half the global population is at risk of DENV infection while ~400 million infections are estimated to occur annually (Beesetti *et al.*, 2014). Yet the global incidence of dengue is considered underestimated (Toan *et al.*, 2015).

Hence, effective vaccines and antiviral agents are needed urgently in order to control its spread (Beesetti *et al.*, 2014 , Hermann *et al.*, 2015). While waiting for the effective vaccines or drugs to emerge, commendable efforts are being made to treat DENV infection using food and nutraceuticals that were reported to either reduce DENV pathogenesis or to improve thrombocytopenia, one of the major consequences of DHF. Nonetheless, studies on the beneficial role of food and

nutraceuticals during DENV infections are grossly lacking (Ahmed *et al.*, 2014).

It is evident that DENV infection stimulates a wide range of biologically active molecules by cells such as B lymphocytes, monocytes, and $CD4^+$ and $CD8^+$ T lymphocytes, mast cells, basophils and platelets (Dhawan *et al.*, 1990, Khanna *et al.*, 1990; Rothman & Ennis, 1999, Lin *et al.*, 2002). These biologically active molecules includes proteases, biogenic amines, cytokines, chemokines, and lipid mediators which are involved in vascular permeability and hemorrhage during DENV infection. Synthesis and secretion of such molecules were also affected by *Carica papaya* based extracts (PBE) and vitamins. The current review highlights the interplay between the biologically active molecules, secretion of which is either affected by DENV infection or by papaya and vitamin consumption. It is shown that the beneficial role of papaya and vitamins in DF is linked to immune modulatory cytokines, enzymes and other biologically active molecules that might either induce thrombopoiesis, prevent the viral entry and replication, decrease the oxidative damage assisted thrombocytopenia, or reduce vascular leakage (Table 1).

Carica papaya based extracts (PBE) and DENV infection

Papaya leaves extract, which is rich in papain, was shown to improve platelet count in dengue patients (Ahmad *et al.*, 2011). Similar extract also increased platelet count in murine animal model (Dharmarathna *et al.*, 2013). *In vitro* induction of IL-6 and stem cell factor (SCF) in response to papaya pulp extracts in human peripheral blood MNCs and stem cells of mesenchymal origin was reported by Aziz *et al.* (2015). Both these cytokines play major roles in megakaryopoiesis and thrombopoiesis (Heinrich *et al.*, 1990, Kaser *et al.*, 2001).

Purified papain, which is one of the major bioactive components in papaya, was also shown to induce IL-6 synthesis in a dose dependent manner in modified mixed lymphocyte culture (Rose *et al.*, 2006). IL-6 produced by smooth muscle cells in the

Table 1. *In vivo* and *in vitro* studies of papaya based extracts and vitamins in relation to the DENV infection

Subjects of Treatment	Mode of Treatment	Results	References
Modified mixed human lymphocyte culture	*In vitro* purified papain	Induced synthesis of IL-6	Rose *et al.*, 2006
Dengue patients	Papaya leaves extract supplement	Improved platelet count	Ahmad *et al.*, 2011
Murine animal model	Papaya leaves extract supplement	Increased platelet count in murine	Dharmarathna *et al.*, 2013
Human γδ T cells and immature dendritic cells	*In vitro C papaya* extracts	Reduced TNF-α production in dose dependent manner	Sagnia *et al.*, 2014
In vitro human peripheral blood MNCs and exfoliated dental pulp stem cells	*In vitro* Papaya pulp extract	Induced synthesis of IL-6 and stem cell factor	Aziz *et al.*, 2015
Dengue patients	1,25(OH)2D & Ca^{2+}supplement	Reduced the severity of DF; increased platelet count	Sanchez-Valdez *et al.*, 2009
Human myelomonocytic (U937) and hepatic (Huh-7) cell cultures infected with DENV type-4	*In vitro* 1,25(OH)2D supplement	Reduced number of DENV infected cells; reduced production of TNF-α, IL-12p70, IL-1b, and IL-6	Puerta-Guardo *et al.*, 2012

tunica media of blood vessels has pro-inflammatory roles. The same cytokine acts as an anti-inflammatory cytokine for its inhibitory effects on TNF-α and IL-1, or activating effects on IL-1 and IL-10. Its secretion is usually induced by stimuli that causes inflammatory responses, including lipopolysaccharides, IFN-γ, TNF-α and -β, viral infections and IL-1 (Heinrich *et al.*, 1990, Hong *et al.*, 2007, Mihara *et al.*, 2012). As a pleiotropic cytokine, IL-6 acts on hepatocytes in the liver, stimulating the increased expression of the otherwise constitutively produced thrombopoietin (TPO), a major inducer of megakaryopoiesis or thrombopoiesis resulting in increased thrombocyte counts (Kaser *et al.*, 2001). IL-6 also increased rate of platelet production by stimulating proliferation of multipotential haematopoietic progenitors (Heinrich *et al.*, 1990). Thus it is possible that PBE might enhance platelet production by inducing IL-6 expression in haematopoietic stem cells and leukocytes. *Carica papaya* extracts was shown to cause dose dependent reduction TNF-α production by γδ T cells and immature dendritic cells (Sagnia *et al.*, 2014).

Papain is a group of major cysteine endopeptidases, present in latex of *C. papaya* unripe fruit, leaf and root which is composed of chymopapain, caricain and glycyl endo-peptidase (Ezike *et al.*, 2009, Azarkan *et al.*, 2003). It is most likely that these proteins can exert their proteolytic enzymes to breakdown the mast cell degranulating polypeptide enzymes responsible for vascular leakage. This proposition might be supported by the observation that the methanolic extract of *C. papaya* elicited angiotensin converting enzyme inhibitory activity. Notably, inhibitory effect of PBE on the plasma angiotensin converting enzyme activity was comparable to that of enalapril, generally used for treatment (Brasil *et al.*, 2014).

Vitamins and DENV infection

Different types of vitamins such as D, E, and C have reported to show promise in small supplementation trials to treat dengue disease (Ahmed *et al.*, 2014). *In vitro* studies using human myelomonocytic cell line U937 and hepatic cell line Huh-7 showed that administration of 1,25(OH)2D can reduce number of infected cells by DENV type-4. It also lowered the production of pro-inflammatory cytokines, namely the TNF-α, IL-12p70, and IL-1b as well as the pleotropic cytokine IL-6. 1,25(OH)2D which also showed the anti-DENV response in a dose dependent manner (Puerta-Guardo *et al.*, 2012). 1,25(OH)2D also down-regulate Toll-like receptors (TLR) expression in human monocytes in a time- and dose-dependent fashion. Consequently p38 and p42/44 phosphorylation (Zhang *et al.*, 2012), and nuclear translocation and activation of NF-kB/RelA were reduced that resulted in reduced proinflammatory cytokine production (Sadeghi *et al.*, 2006). Patients treated with related with 1,25(OH)2D and calcium were shown to reduce the severity of DF (Sanchez-Valdez *et al.*, 2009). Vitamin E supplement (400 mg/day) along with the standard treatment resulted in faster and greater increase in platelet count compared to the control group of patients who received similar treatment without Vit E supplement. Improvement in circulatory platelet count was also observed in DF patients given Vitamin E (400 mg/day) and Vitamin C (3x, 500 mg/day) for 8 days. Thrombocytopenia was seen in infants supplemented with vitamin A, a known antioxidant, at a higher dose 62000 IU/day for 80 days (Perrotta *et al.*, 2002). Moreover, higher level of circulatory vitamin A was seen in patient suffering from DENV (Mawson, 2013).

Usually immune cells express vitamin D receptors (VDR) to which vitamin D binds and subsequently activates the responsive genes that regulate immune response against pathogens (Provvedini *et al.*, 1983, Kongsbak *et al.*, 2013). Association of vitamin D deficiency with autoimmune disease (Agmon-Levin *et al.*, 2013) and viral infection susceptibility especially to influenza has been reported (Beard *et al.*, 2011, Cannell *et al.*, 2006). Frequency of vitamin D deficiency is very high in patient suffering from HIV as well (Allavena *et al.*, 2012). Notably, proinflammatory cytokines are involved in the immunopathogenesis of severe dengue disease (Aziz *et al.*, 2015, Soundravally *et al.*, 2014). Thus, one of the possible mechanism

of 1,25(OH)2D protection against DENV infection could be an expression of proinflammatory cytokines.

Vitamin E, more particularly α-Tocopherol, is a lipid-soluble antioxidant which responds to oxidative stress through the glutathione peroxidase pathway (Wefers & Sies, 1988), hence, the thrombocytopenia, the most fatal consequences of DENV infection, which is often linked with oxidative stress (Soundravally *et al*., 2008) could be minimized, if not prevented, by vitamin E administration.

Platelet activating factor (PAF) and activated PAF-receptor (PAFR) on endothelial cells and leukocytes are involved in increased vascular permeability (Souza *et al*., 2009, Jeewandara *et al*., 2015) observed during DENV infection. The $PAFR^{-/-}$ mice infected with DENV showed decreased thrombocytopenia, hemoconcentration, decreased systemic levels of cytokines, and delay of lethality (Souza *et al*., 2009). Vitamin C supplementation was found to prevent the accumulation of PAF-like lipids, and leukocyte adhesion to the vascular wall and formation of leukocyte-platelet aggregates in smokers (Lehr *et al*., 1997). Besides, PAF/PAFR activates p38 MAPK pathway (Marques *et al*., 2002, Chen *et al*., 2005) that increases the production of proinflammatory cytokines (Nahirnyj *et al*., 2013). Hence, the vitamin C supplement in DENV infected patients might be proven beneficial.

CONCLUSION

The current knowledge on the pathogenesis of DENV infection and cytokine interplay gives hope that certain food and nutraceuticals might help to improve DENV infected conditions. Among the potential candidates, *Carica papaya* based extracts and vitamins showed beneficial role in DENV infected patients (Figure 1). For example, vitamins might stimulate T_{H1} to secrete IFN-α that in turn might block DENV entry and replication (Figure: B3, B4, C3). Vitamin could also help preventing thrombocytopenia (Figure: D2). Again, PBE might activate hematopoietic stem cells and DC to secrete TNF-α and IL-6, those which in turn might induce the liver to synthesize TPO hence thrombopoiesis can be enhanced in DF patients (Figure: D3, D4, E4, E5). Furthermore, proteolytic enzymes in PBE could digest mast cells degranulating enzymes thus prevent vascular leakage (Figure: B1, C1, D1).

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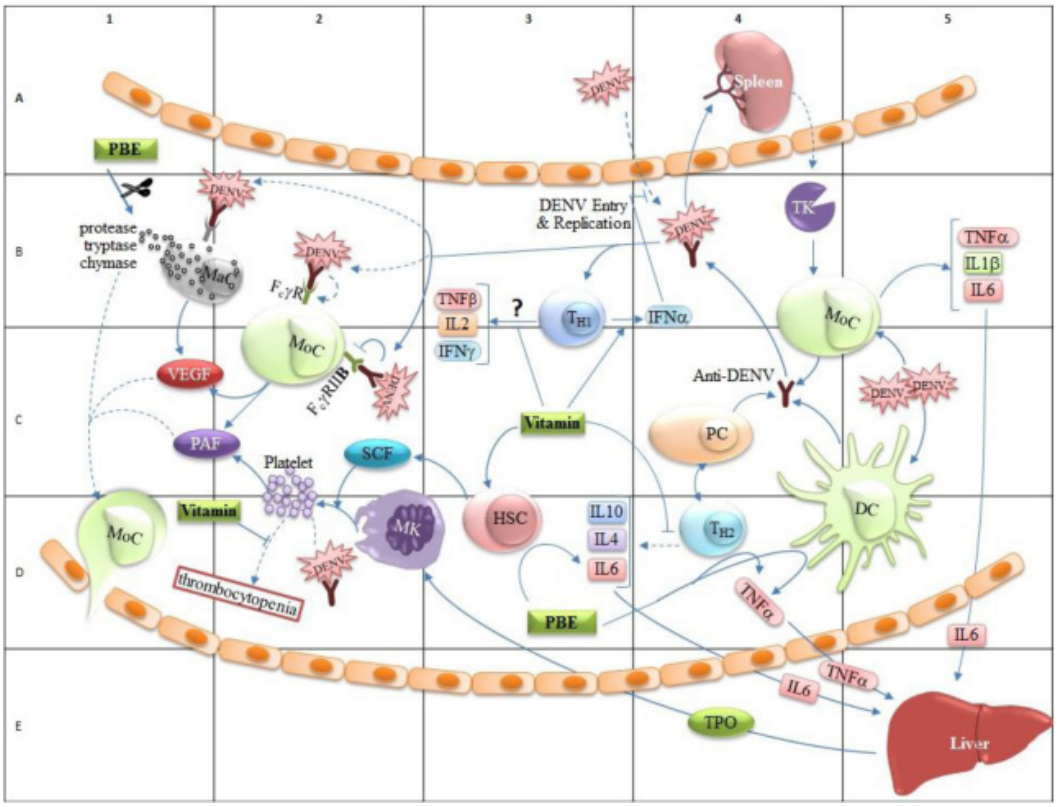

Figure 1. Potential mechanism of food and nutraceuticals such as vitamins and papaya based extracts (PBE) to improve DENV infected conditions.

DENV pathogenesis and body immune response involves variety of immune cells such as monocytes (MoC), mast cells (MaC), dendritic cells (DC), plasma cells (PC), hematopoietic stem cells (HSC), megakaryocytes (MK), platelets, and helper T cells (T_{H1} and T_{H2}). At the same time each type of cell is involved in synthesis and secretion of variety of cytokines, growth factors and interleukins. These biological factors are involved either in DENV pathogenesis such as vascular leakage and thrombocytopenia . Food and nutraceuticals might help to improve the conditions of vascular leakage and thrombocytopenia. For example vitamins prevent thrombocytopenia while PBE might help to degrade protease, tryptase and chymase secreted by MaC responsible for vascular leakage. Vitamins, PBE, and crab might help to induce proinflammatory cytokines thus improve DENV infected conditions. [arrow head: stimulatory or activating responses; arrow bar: inhibitory or preventing responses; arrow head with dotted lines: pathogenic mechanisms that can be controlled by either PBE or vitamins]. The figure is divided into grids where columns are numbered from 1 to 5 and rows are labelled as A-E. Description of a particular event is indicated using the grid label and number such as A3, C4 and E1.

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