

# Biochemical Role of Zinc in Dengue Fever

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## Abstract

**Background:** Zinc is an essential trace element that has been shown to play a crucial role in viral infections. As a part of RNA polymerases, zinc helps in the replication of many viruses. Cholinesterases are low-grade inflammatory markers that have zinc-dependent carboxypeptidase activity. Plasma butyrylcholine esterase is an indicator of dietary zinc level. Matrix metalloproteinases (MMP) that participate in inflammatory processes and contribute to tissue remodeling and repair are zinc containing endopeptidases. The present study attempts to establish the association of zinc with these enzymes in dengue. **Materials and Methods:** Plasma zinc, butyrylcholinesterase (BChE), and erythrocyte acetylcholinesterase (AChE) were estimated spectrophotometrically in 100 dengue patients and 50 healthy controls. MMP9 was determined using ELISA. **Results:** Plasma zinc was markedly lower in dengue fever patients compared to healthy controls ( $P < 0.05$ ). Both BChE and AChE decreased significantly in these patients ( $P < 0.001$ ) compared to healthy controls. There was an elevation of MMP9 in dengue, although the increase was statistically insignificant. Cholinesterases and MMP9 correlated positively with zinc in controls and dengue, although the correlation was statistically insignificant. **Conclusion:** The study highlights the role of zinc in the pathology of dengue, as it is essential for the maintenance of activities of cholinesterases and MMP9 that are involved in anti-inflammatory and tissue remodeling processes. Further, prompt administration of micronutrients like zinc may reduce the adverse manifestations and morbidity in dengue.

**Keywords:** Acetylcholinesterase, butyrylcholinesterase, dengue, matrix metalloproteinases, zinc

## INTRODUCTION

Dengue is a mosquito borne infection that has become a major public health burden in Southeast Asian countries. Although the pathogenesis of dengue fever remains elusive, the degree of disease severity is multifactorial which depends on factors like viral virulence, the immune response to infection, host factors including genetic and nutritional status.<sup>[1]</sup>

Malnourished children with dengue had higher morbidity and mortality suggesting the role of the nutritional factors in its pathogenesis.<sup>[2]</sup> Evidence from clinical studies on dengue fever has indicated extensive immune activation with the overproduction of cytokines.<sup>[3]</sup> However, zinc deficiency can result in inefficient defense against infections by impairing both innate and adaptive immunity and creating an imbalance of pro- and anti-inflammatory cytokines leading to apoptosis of T-cell precursors.<sup>[4]</sup> Zinc is a component of more than 300 enzymes involved in catalysis, redox regulation, and signaling. Cholinesterases namely acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) found in liver, erythrocyte membranes, platelets, lymphocytes, and to a lesser extent in

nervous tissue have zinc-dependent carboxypeptidase activity. Plasma BChE activity is sensitive to dietary zinc status and reflects the nutritional status of the patients.<sup>[5]</sup> Matrix metalloproteinase (MMP) is a zinc containing endopeptidase that has a role in cell proliferation and tissue repair.<sup>[6]</sup> These enzymes are produced by fibroblasts, endothelial cells, hemopoietic cells, and other inflammatory cells. MMP9 is capable of digesting endothelial glycocalyx and enhances endothelial permeability resulting in plasma leakage in dengue.<sup>[7]</sup> Several MMPs are upregulated by dengue virus infection.<sup>[8]</sup> To our knowledge, this is the first study that reports the relationship between zinc and MMP9, BChE, and AChE in dengue infection.

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## MATERIALS AND METHODS

The research was conducted ethically in accordance to the Declaration of Helsinki on Biomedical research involving human subjects and was approved by the Institution Ethics Committee (IEC KMC MLR 12–17/252) and informed consent was obtained from all the subjects.

### Study participants and data collection

A cross sectional study was conducted in the Clinical Biochemistry laboratory of a tertiary care hospital. Blood samples were collected 48–96 h after the onset of fever from 100 freshly diagnosed serologically positive dengue patients with the age group ranging from 18 to 50 years. The data obtained were compared with that of 50 age- and sex-matched normal individuals. Exclusion criteria included individuals with other infectious diseases such as otitis, typhoid, malaria, and AIDS. Patients with disorders such as diabetes mellitus, rheumatoid arthritis that may affect plasma zinc levels were not included. Smokers, subjects on minerals and vitamin supplements were also excluded from the study. Baseline demographic characteristics of patients including sex and age were recorded along with clinical manifestations.

The serological test for IgM was done using the ELISA kit. Fasting blood samples were collected in heparin vacuum tubes by venipuncture and centrifuged at 3000 rpm for 10 min. The plasma separated was used for biochemical analysis. Plasma zinc was estimated using nitro-PAPS as a chromogen.<sup>[9]</sup> Plasma zinc reacts with nitro-PAPS in an alkaline medium to form a purple colored complex which was read at 570 nm spectrophotometrically in a semiauto analyzer. Plasma BChE and red blood cell AChE were determined by their action on acetylthiocholine to form thiocholine that reacts with Ellman's reagent to give yellow nitrothiolate ion, which was measured at 412 nm.<sup>[10]</sup> Plasma MMP9 was quantified by sandwich immunoassay using commercially available ELISA kits.<sup>[11]</sup>

### Statistical analysis

Data were analyzed using independent Student's *t*-test using SPSS version 20 (BM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp. IBM Corp). Pearson correlation was done to find out the association of zinc with the enzymes.  $P < 0.05$  was considered statistically significant.

## RESULTS

Table 1 depicts mild impairment of liver function in dengue patients and [Table 2] indicates the clinical signs. Plasma zinc was significantly low in dengue patients ( $P < 0.05$ ) compared to normals. There was a marked decline in plasma butyryl cholinesterase and acetyl cholinesterase in dengue patients ( $P < 0.001$ ). However, the increase in plasma MMP9 was marginal in these patients compared to controls [Table 3]. Cholinesterases and MMP9 showed a positive correlation with plasma zinc both in normals [Figure 1a-c] and dengue patients [Figure 1d-f].

**Table 1: Baseline data and liver function tests in normal and dengue patients**

	Mean ± SD		P
	Control (n=50)	Dengue (n=100)	
Age	32.33±7.8	43±13	0.58
Male:Female	18:32	29:71	0.26
ALT (IU/L)	28.5±2.19	95±8.96	0.03
AST (IU/L)	41.41±10	99±12	0.06
ALP (IU/L)	68±15	104±70	0.001
Albumin (g/dL)	4.46±0.21	3.75±0.41	0.05
Total protein (g/dL)	7.46±0.33	6.53±0.73	0.05
Total bilirubin (mg/dL)	0.89±0.15	1.21±0.46	0.05

n=Number of samples. SD: Standard deviation, ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase

**Table 2: Clinical features in dengue patients**

Symptoms	Number of patients
Sore throat	31
Cough	53
Rash	16
Myalgia	34
Diarrhea	32
Nausea	90
Head ache	76
Abdominal pain	64

Figures indicate number of patients

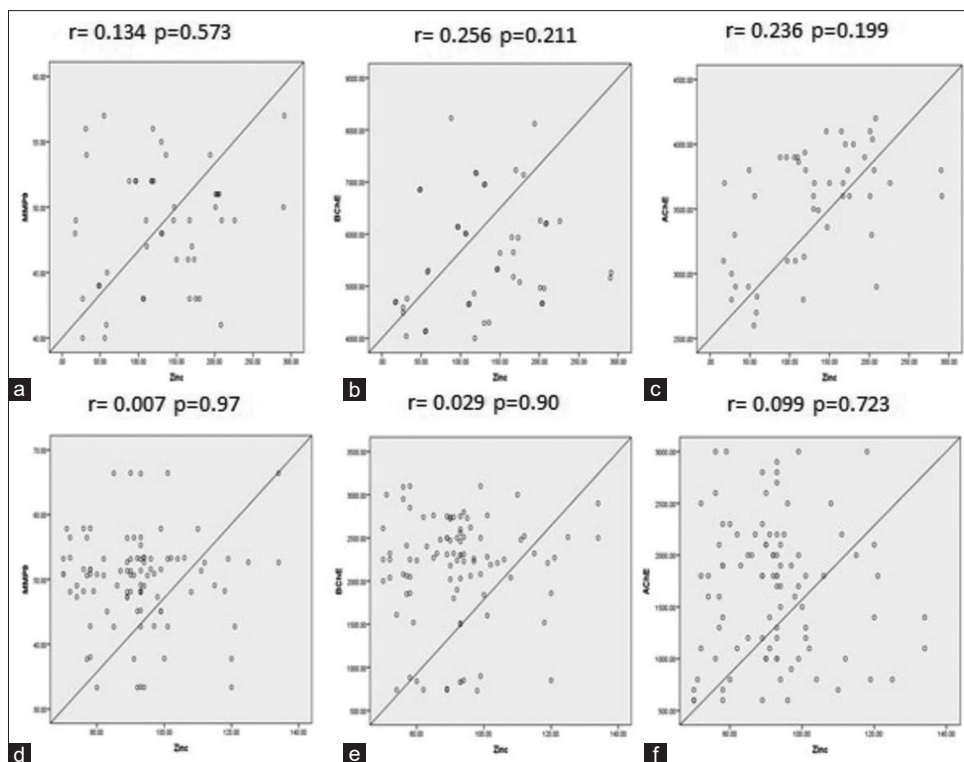
**Table 3: Comparison of zinc and enzymes in dengue and controls**

	Mean ± SD		P
	Control (n=50)	Dengue (n=100)	
Zinc (µg/dL)	134±14	93±17	0.05
BChE (U/L)	5611±107	2190±130	0.001
AChE (U/L)	3606±309	1700±109	0.001
MMP-9 (ng/mL)	49±8.8	53±6.55	0.20

n: Number of samples. SD: Standard deviation, BChE: Butyrylcholinesterase, AChE: Acetylcholinesterase, MMP: Matrix metalloproteinases

## DISCUSSION

Zinc is the most abundant trace element after iron in the human body. There is a strong evidence that links its deficiency with infectious diseases such as HIV, malaria and tuberculosis.<sup>[12]</sup> Although its participation in numerous cellular functions is now well established, this study attempts to explore other roles of zinc in dengue. In this study, patients with dengue had significantly low plasma zinc compared to healthy individuals. Multiple studies have reported low serum zinc in children with dengue fever.<sup>[3,13]</sup> Lack of specialized zinc storage system mandates sufficient daily intake of zinc to avoid diseases. Zinc is a common micronutrient that has an immunomodulatory function. Zinc deficiency adversely affects the macrophages which dysregulate intracellular killing, cytokine production and phagocytosis. Apoptosis is potentiated by zinc deficiency accompanied by



**Figure 1:** Scatter plots illustrating positive correlation between Zinc and matrix metalloproteinase 9, butyrylcholinesterase, acetylcholinesterase in control (a-c) and in dengue (d-f)

a decrease in T-helper cell function resulting in increased susceptibility to infection.<sup>[4]</sup> Zinc is an acute phase reactant and during the early phase of dengue infection, the inflammatory reaction causes translocation of zinc from plasma into the liver to prevent oxidative damage to the liver.<sup>[14]</sup> This redistribution may create transient hypozincemia which may indirectly protect the host cells by inducing an antiviral environment.<sup>[15]</sup> Widagdo<sup>[16]</sup> reported higher plasma zinc in patients with dengue hemorrhagic Grade III fever than those with Grade I and Grade II. Zinc is likely to play a prominent role in the RNA polymerase activity of the dengue virus. The enzyme has two zinc binding pockets in one of the domains and zinc contributes to its structural stability.<sup>[17]</sup> About 10% of human proteins have the potential to bind with zinc, indicating the involvement of zinc in numerous physiological processes. In the current study, BChE and AChE were significantly low in dengue patients. Low cholinesterase activity could be due to decreased dietary intake or/and reduced plasma zinc concentration. Plasma BChE is an indicator of dietary zinc status.<sup>[3]</sup> Further, serum BChE was found to be low in various clinical conditions like inflammation, injury and infections.<sup>[18]</sup> Hepatic inflammation and dysfunction is a crucial feature seen in dengue infection<sup>[19]</sup> which would have led to decreased BChE synthesis. There are several reports on the involvement of zinc in chronic inflammatory diseases such as diabetes mellitus, cancer, and neurodegenerative diseases.<sup>[12]</sup> Inflammation was associated with reduced plasma zinc levels in these diseases. Moreover, there was a positive correlation between zinc and cholinesterases in the current study, which will justify the role of zinc in enzyme activity, thereby inflammation

in dengue. Dysfunction of the liver may be due to direct viral attack on hepatocytes or an adverse effect of dysregulated host immune response against the virus.

Further, one of the prominent physiological functions of zinc is in the regulation of permeability in vascular endothelial cells. Hence, hypozincemia may contribute to plasma leakage, a characteristic feature of dengue hemorrhagic fever. The results of the present study showed an apparent elevation in MMP9 levels in dengue patients. On the contrary to our findings, Voraphani *et al.*<sup>[20]</sup> reported that MMP9 levels decreased during febrile and toxic stages of dengue virus infection. However, MMPs are capable of digesting endothelial glycocalyx contributing to increased permeability leading to plasma leakage in dengue. Several earlier reports associate the severity of the disease with the increase of MMP9.<sup>[7]</sup> Dengue infection increased endothelial permeability through the overproduction of MMPs by immature dendritic cells of blood and macrovascular endothelial cells.<sup>[8]</sup> The nonstructural protein of the dengue virus induces MMP9 secretion by leukocytes.<sup>[8]</sup> In the current study, the positive correlation of MMP9 with zinc establishes that MMP is dependent on zinc for its proteolytic activity.

## CONCLUSION

The study highlights the role of zinc in the pathology of dengue and also emphasizes its anti-inflammatory and tissue remodeling functions. Further, prompt administration of micronutrients like zinc may reduce the adverse manifestations and morbidity in dengue.

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## Conflicts of interest

There are no conflicts of interest.

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