Research article

Biochemical role of zinc in vivax malaria

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ABSTRACT

Introduction and Aim: Zinc is a micronutrient that has a significant immune modulatory function, its deficiency is known to increase the risk of plasmodium infection in humans Although inflammatory processes contribute to the elimination of plasmodium, persistent inflammation is the underlying cause of malaria pathology. Cholinesterases are carboxypeptidases that are zinc dependent and are the indicators of low-grade inflammation. Plasma butyryl cholinesterase, is a biomarker of dietary status of zinc. Matrix metalloproteinases (MMP) are endopeptidases that contain zinc which take part in inflammatory reactions. They participate in remodeling of extracellular matrix and aid tissue repair. The current investigation aims to study the association of these metalloproteins with zinc in patients with vivax malaria.

Materials and methods: Plasma zinc, butyrylcholinesterase (BChE) and erythrocyte acetylcholinesterase (AChE) were estimated spectrophotometrically in 100 vivax malaria patients and 50 healthy subjects. MMP9 was determined using ELISA.

Results: Plasma zinc was markedly lower in malaria patients compared to healthy controls ($p<0.05$). Both BChE and AChE decreased significantly in malaria ($p<0.00$) compared to healthy subjects. There was an elevation of MMP9 in malaria, although the increase was statistically insignificant. Cholinesterases correlated positively with zinc in controls and malaria. MMP9 showed a significant positive correlation with zinc in malaria patients ($r=0.405$, $p=0.05$).

Conclusion: The study highlights the role of zinc in the pathology of malaria as it is essential for the maintenance of several enzyme activities. Further, prompt administration of micronutrients like zinc may reduce the inflammation related morbidity in malaria.

Keywords: Acetylcholinesterase; butyrylcholinesterase; malaria; MMP 9.

INTRODUCTION

Malaria contributes to the global infectious disease burden with very high morbidity and mortality, especially in coastal South India. The parasite, which resides in the liver and erythrocytes will be invisible to the immune surveillance; however, the spleen destroys the infected circulating RBCs. Zinc is a micronutrient that has a significant immune modulatory function (1). In human beings its deficiency is known to enhance the risk of plasmodium infection and susceptibility to viral infections (2). Liver, erythrocyte membranes, platelets, lymphocytes, and nervous tissue are the sites of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) which are carboxypeptidases that are zinc-dependent (3). Plasma BChE activity is highly susceptible to change with dietary zinc intake and reflects the nutritional condition of the individuals (4). Matrix metalloproteinases (MMPs) are zinc endopeptidases which play a pivotal role in tissue repair and cell division (5). MMPs are expressed in inflammatory cells, fibroblasts, hematopoietic cells, and endothelial cells. Unlike other MMPs, MMP9 is an inducible enzyme that can be activated by hemin, a core component of malaria pigment. Therefore, MMP9 gains importance in malarial research. Cytoadherence of parasitized red cells to vascular endothelium contributes to vascular damage and inflammatory response in malaria. Therefore, MMP9 may have a predominant role in malaria where RBC turnover is very high. Hence, the present study is undertaken to demonstrate the role of zinc in malarial pathology by evaluation of zinc dependent plasma enzymes.

MATERIALS AND METHODS

This study was approved by the Institutional Ethics Committee (IEC- KMC MLR 12-17/252) and informed consent was taken from the subjects enrolled for the study. The study population included 100 symptomatic, freshly diagnosed RMAT positive vivax malaria patients between the age of 15-50 years. The clinical symptoms of the patients on preliminary examination were headache, malaise, periodical attacks of chills and fever. None of the patients recruited for the study were under antimalarial therapy or had a known history of previous malarial attack. Fifty age and sex matched healthy subjects of the same socio-economic status were chosen for the control group. Exclusion criteria included individuals with other sources of infection like otitis, typhoid, dengue, and chronic illness like AIDS. Patients with systemic illness like rheumatoid arthritis, diabetes which may alter zinc levels in blood were also excluded. Patients

exposed to pesticides and who were on micronutrients supplementation were not considered for the study.

Fasting blood samples were collected in heparin vacuum tubes by venipuncture and centrifuged at 3000 rpm for 10 minutes. The plasma separated was used for biochemical analysis. 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 semi auto analyzer (6). Plasma BChE and RBC AChE were determined by their action on acetylthiocholine to form thiocholine that reacts with Ellman's reagent to give yellow nitro thiolate ion, which was measured at 412 nm (7). Plasma MMP9 was quantified by sandwich immunoassay using commercially available ELISA kits (8). Routine biochemical and hematological investigations were carried out on all the subjects. Data were analyzed by independent student t-test using SPSS version 20. Pearson's coefficient was calculated to assess the correlation of zinc with the enzymes. $p<0.05$ was considered statistically significant.

RESULTS

Demographic, hematological, and biochemical parameters as depicted in table 1 were not significantly different in normal and malaria patients. In patients with malaria plasma zinc was significantly reduced compared to healthy subjects ($p<0.05$). Cholinesterases found both in erythrocytes and plasma decreased significantly in patients with malaria ($p<0.001$). There was an apparent increase in plasma MMP9 in malaria patients compared to normal individuals (Table 2).

Plasma zinc correlated positively with BChE, AChE, and MMP9 in both healthy individuals and malaria patients. Further, the correlation of MMP9 with zinc in malaria was statistically significant ($r=0.405$, $p=0.05$) (Table 3).

Table 1: Demographic and baseline data in the study groups

Variables	Control (n=50) Mean ± SD	Malaria (n=100) Mean ± SD	p value
Age	32.33 ± 7.8	31.9 ± 13.9	0.58
Male: Female	32:18	69:31	0.26
Hb (g/dl)	13±0.40	12 ± 0.61	0.80
RBC (10^6/µL)	5.0 ± 0.23	4.2 ± 0.32	0.65
WBC (10^3/µL)	6.5 ± 0.21	6.2 ± 0.33	0.77
Platelet (10^3/µL)	105 ± 50	97 ± 11	0.41
ALT(IU/L)	28.5 ± 21.9	35.92 ± 2.04	0.18
Albumin(g/dL)	4.46 ± 0.21	4.20 ± 0.41	0.15
Protein total(g/dL)	7.36 ± 0.33	7.27 ± 0.53	0.19
Bilirubin total(mg/dL)	0.89 ± 0.15	2.07 ± 0.46	0.02*
Bilirubin Direct(mg/dL)	0.26 ± 0.11	0.20 ± 0.05	0.57

n=number of samples, *Significant

Table 2: Comparison of zinc and enzymes in study groups

Variables	Control (n=50) Mean ± SD	Malaria (n=100) Mean ± SD	p value
Zinc (µg/dL)	134 ± 14.5	91± 11.5	0.05
BChE (U/L)	5611 ± 107	2732 ± 310	0.001
AChE (U/L)	3606 ± 309	1700 ± 109	0.001
MMP9 (ng/mL)	49.05± 8.8	52.57± 1.71	0.20

n = number of samples

Table 3: Correlation coefficients of zinc with enzymes in malaria and controls

Variables	Control		Malaria	
	r	p	r	p
BChE	0.256	0.211	0.052	0.817
AChE	0.236	0.199	0.099	0.723
MMP9	0.134	0.573	0.405	0.05*

*Significant

DISCUSSION

This study demonstrated a significant decline in plasma zinc levels in patients with vivax malaria compared to normal individuals, which agrees with one of the earlier studies (9). Zinc concentration was four-fold higher in plasmodium infected red cells than the uninfected ones indicating the need for acquisition of zinc atoms into RBCs by the parasite before hemolysis can occur (10). Many pathogens need zinc for adherence to mammalian cells. Plasmodium uses up host trace elements like iron, copper and zinc for its metabolic pathways leading to the deficiency of these nutrients in the host culminating in increased

morbidity in these patients (11). Zinc supplementation reduced malaria morbidity in children in one of the community-based studies (12). However, Muller *et al.,* (13) did not find any effect of zinc supplementation on morbidity due to Plasmodium falciparum infection. The plasmodium genome is regulated by zinc finger transcription factors. Thus, zinc acts as a signaling mediator that may be linked to disease progression (14). Zinc being an antioxidant micronutrient may be partially used up by the malarial parasite to fight against the respiratory burst in the host, leading to a decrease in plasma zinc in malaria patients.

Further, the host releases inflammatory cytokines as an early immune response to plasmodium infection which may result in hypozincemia. Although, generally plasma zinc decreases in infectious diseases, it is now clear that the decrease may not be due to dietary inadequacy but as a part of defense strategy Moreover, zinc may be redistributed from plasma to lymphocytes and liver during the acute phase of several infections (15). Even though zinc depletion may impair host immune cells functions, hypozincemia may hinder the proliferation of malarial parasites in acute conditions. Hence a moderate deficiency of zinc in the infected patients may be a nutritional adaptation to endemic diseases (11).

In the current study, plasma cholinesterases decreased significantly in malaria. Since BChE has zinc-dependent carboxypeptidase activity, low levels in malaria could be due to low plasma zinc level or decreased dietary intake. Moreover, serum BChE was found to be lower in injury, inflammation, and several other acute phase conditions like infections (16), which justifies the findings of the present study. Further, bilirubin overload on hepatocytes, a source of BChE may be another cause for its decrease in malaria. AChE, an erythrocyte membrane bound protein, may be expected to be lower in malaria patients due to the enhanced rate of destruction of circulating blood cells.

High plasma MMP9 observed in malaria patients proves its contribution in the process of tissue remodeling. It is being overexpressed even in other infectious diseases like meningitis, trypanosomiasis, Leishmaniasis, and dengue (17, 18). Serum MMP8 was seen to be elevated in patients with both severe and complicated malaria, but MMP9 was not different from healthy subjects (19), which is contradictory to the results of the current study. MMP9 are proteolytic enzymes that are mainly involved in the degradation of extracellular components and regulate inflammatory processes and modulate the expression of pro-inflammatory molecules like cytokines and chemokines during infections. Further, parasite toxins cause activation of innate immunity and release of inflammatory mediators. Cytoadherence of parasitized red cells to the vascular endothelium of vital organs like the brain, liver, kidney may disturb the metabolism that leads to tissue damage and release of MMP9. Brown HC et al (20) linked increase in plasma MMP9 level to loss of blood-brain barrier integrity in both bacterial and tuberculous meningitis but not in falciparum malaria. In malaria, the trophozoite laden RBCs or hemozoin fed monocytes displayed increased MMP9 activity and increased mRNA expression. TNFα production is enhanced by hemozoin produced by malarial parasites, which in turn increases the MMP9 gene expression (21). MMP9 single nucleotide polymorphism was protective against placental malaria, which suggests a possible role of the enzyme in decreasing the susceptibility to infection. Increased expression of MMP9 has been observed in acute malaria. Though inflammatory response contributes to the pathophysiologic damage, increased MMP9 enhances parasite elimination in malaria (22). Moreover, in the present study, a significant positive correlation between zinc and MMP9 underlines the fact that the enzyme activity is dependent on plasma zinc status and may act as a biomarker of malaria.

CONCLUSION

The result of this investigation highlights the contribution of zinc in the maintenance of enzyme activities that have a role in inflammatory processes in malaria. Hence, timely administration of micronutrients like zinc may reduce the inflammation related morbidity in malaria.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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