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Biochemical Association between Essential Trace Elements and Susceptibility to Malaria in Outbred Mice after Inhibition with Dexamethasone or Induction with Lipopolysaccharide

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Abstract

The aim of this study was to evaluate the effects of the parasitaemia, pathological states on the concentration of trace elements (iron, copper, zinc) during malaria infection. Malaria is one of the commonest parasitic diseases affecting humans and animals in the world it is a parasitic disease which is common in tropical and subtropical regions of the world. Some trace elements that are also essential for malaria parasite. The aim of this work was to assess serum titers of Cu, Zn and Cu/Zn ratio, an indicator of oxidative stress during malaria. Several enzymes that contribute to immune system responses require zinc and copper as trace elements. We examined zinc and copper levels in malaria infected NMRI mice. Serum Zn and Cu were determined by flame atomic absorption spectrophotometry. ANOVA and paired t-test analysis were used to determine the association trace elements changes. Higher Cu levels were found in inhibitor (Dexa methasone) and higher Zn

levels were found in inducer (Lipopolysaccharide) and Cu/Zn ratios were increased in inhibitor (Dexamethasone). We conclude that concentrations of essential trace elements vary during activity.

Keywords: Malaria, Lipopolysaccharide, Dexamethasone, Microelements

Introduction

Malaria is one of the commonest parasitic diseases affecting humans and animals in the world [1]. It is an infection disease which is caused by protozoan parasites in genus *Plasmodium*. It is transmitted by a species of female Anopheles mosquitoes. It is one of the most important infection diseases in tropical and subtropical regions [2]. Malaria is a complex disease that varies widely in epidemiology and clinical manifestations in the different parts of the world. In spite of enormous effort to control it, malaria still represents one of the most important, parasitic diseases of human worldwide [3]. In spite of the numerous control strategies, including vector control, parasite treatment and environmental sanitation, there has been no reduction in the number of infected cases. During the past few decades, it has become clear that malaria eradication in endemic areas is unlikely to be achieved by vector control and chemotherapy alone; therefore, more focus has been made on neuroprotective agents, especially vaccines. Despite intensive research efforts, no vaccine against malaria that is both effective and suitable for mass production is yet available [4].

Trace elements are needed for many metabolic and physiological processes in the human body (Mertz, 1988).Trace metals, including Zn and Cu, are directly involved in metabolic processes critical to cell differentiation and replication [5]. Zinc (Zn) and Copper (Cu) are the essential elements that play a crucial role in the immune system. These trace elements act as cofactors for antioxidant enzymes involved in the destruction of toxic free radicals produced in the body. The serum levels of antioxidants vary in many diseases including malaria. These alterations are part of defense strategies of organism and are induced by different cytokines [6-7]. The immune function is deftly synchronized by zinc, since both increased and decreased zinc levels result in a disturbed immune function [8]. Zn is an essential trace element with cofactor functions in a large number of proteins of intermediary metabolism, hormone secretion pathways and immune defense mechanisms. Zn, an essential trace element, is important in numerous critical biochemical processes since it's a cofactor in about 200 metalloenzymes including Cu/Zn-superoxide dismutase, a critical cytoplasmic antioxidant enzyme [9]. One of the main clinical manifestations associated with Zn deficiency is increased susceptibility to infectious diseases [5]. Cu could be a potential inducer of LDL oxidation**.** On one hand, Cu has the ability to involve in preventing oxidative injury. In addition, caeruloplasmin, a multifunctional protein which contains most of the Cu in blood, is thought to possess antioxidant functions, which could be beneficial in resisting disease Copper is essential also for maintaining the strength of the skin, blood vessels,

epithelial and connective tissues. It plays a role in production of haemoglobin, myelin and melanin. Since various controversial results on metals are reported in many reported studies, it is difficult to determine the role of these metals in malaria patients [10].

Lipopolysaccharide (LPS) as an inducer and dexamethasone (DEX) as an inhibitor of the immune system are used in this study [2]. LPS is in the outer membrane of negative bacteria such as *Haemophilus*, *E.coli*, *Pseudomonas*, *Salmonella* and *Shigella*, and composed of two main parts lipid and polysaccharide, and his name is bacterial endotoxin. Immunogenicity of LPS is due to its polysaccharide form. LPS is one of the main components of the outer wall of Gram-negative bacteria, which has a molecular weight of 2 - 20 kDa. Also that's a phosphorylated lipid portion of the molecule forming the outer membrane and a hydrophilic polysaccharide matrix is formed. It also used generally as a "standard stimulus" for the induction of inflammation or fever in laboratory animals and can lead to symptoms such as high blood pressure, metabolic acidosis, increased glucose and potassium [26]. Dexamethasone (DEX) is from glucocorticoid hormone families that have anti-inflammatory properties. Glucocorticoids are potent drugs that can be used widely inflammatory disorders such as asthma, allergies, infections, autoimmune diseases [2]. Trace metals are reviewed briefly in this study of trace metals including copper and zinc in the serum of malaria patients in comparison to normal animal model.

Materials and Methods

Animals and groups: The experiments were performed four groups considering time**,** NMRI mice (supplied by the Karaj Laboratory Animal Unit, Pasteur Institute of Iran) were used in this study. The initial body weight was 19.2±1.3 g (mean ± standard error of mean, SEM) and mice were housed at room temperature (20-23°C) on a 12-h light and 12-h dark cycle, with unlimited access to food and water. Experiments with animals were done according to the ethical standards formulated in the Declaration of Helsinki and measures taken to protect animals from pain or discomfort. It has been approved by Ethical Committee of the Pasteur Institute of Iran, in which the work was done.

Malaria parasites: The rodent malaria parasite used in this study was the Plasmodium berghei which represents a third of rodent malaria species. The infectivity of the parasites was maintained. Serum Cu and Zn were determined by flame atomic absorption spectrophotometry (FAAS, Thermo Jarrel Ash, Germany) [11]. Analyses were made with the Student's *t* test, using Graph Pad Prism Software (Graph Pad, San Diego, CA, USA).

Results

Statistically significant differences in serum Zn were observed among groups. Zn decreased in test group compared to control group in DEX mice ($P<0.0003$) and in LPS group ($P<0.33$) (Figure 1). Statistically significant differences in serum Cu

were observed among groups. Cu increased in test group compared to control group in DEX mice (P<0.02) and in LPS group (P<0.002) (Figure 2). Statistically significant differences in serum Cu/Zn ratio was observed among groups. Cu/Zn ratio increased in test group compared to control group in DEX mice (P<0.1) (Figure 3).

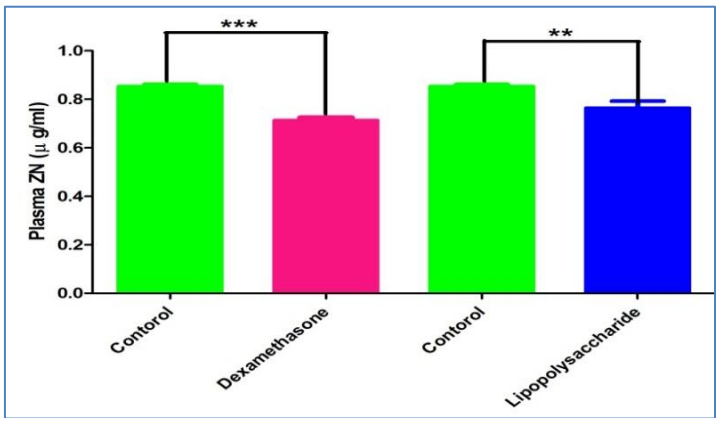

Figure 1. Plasma Zn values after inoculation with DEX as inhibitor and LPS as inducer of immune system

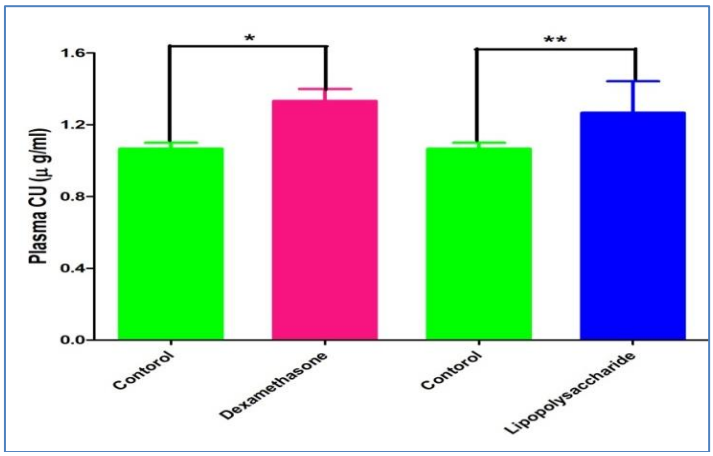

Figure 2. Plasma Cu values after inoculation with DEX as inhibitor and LPS as inducer of immune system

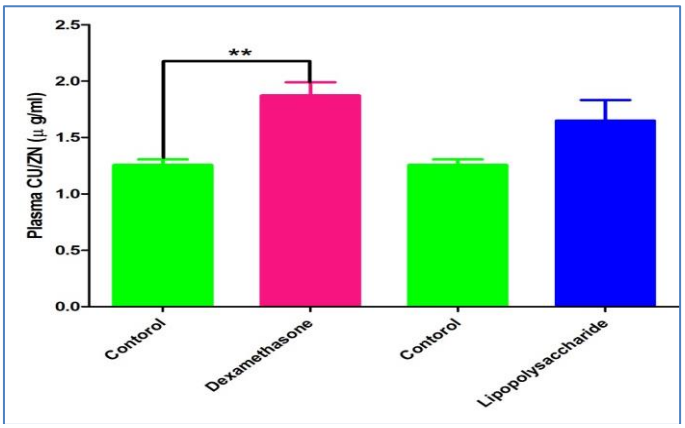

Figure 3. Plasma Cu/Zn ratio values after inoculation with DEX as inhibitor and LPS as inducer of immune system

Discussion

The involvement of trace elements in the protection or exacerbation of malaria has been a subject of numerous studies, The immunomodulatory properties of zinc are known [12-13]. Zn and Cu are involved in numerous aspects of cellular metabolism [14]. They are required for the catalytic function of several enzymes, play essential roles in immune function and act as antioxidants [15-16]. Maintaining a proper balance of copper and zinc is important as excess Zn impairs Cu absorption [17]. The ratio of Cu/Zn is clinically more important than the concentration of either of them. Zn is a cofactor in more than 300 enzymes influencing various organ functions having a secondary effect on the immune system [18]. It also influences the function of immune-stimulants used in the experimental systems. It is also known that Cu plays a role in the biochemical pathways of mammals. Apart from these well-known functions, Zn and Cu also play a critical role in host-pathogen interactions. Zn is required for each step of cell cycle in microorganisms [19]. Cu is an essential component of Cu / zinc superoxide dismutase; an enzyme in the erythrocytes essential for host defense as well as parasite growth [20]. The metabolic pathways of plasmodium require several enzyme cofactors such as iron sulphur clusters [21].

There are several publications representing microelements in infections. Amini *et al*. (2008) reported no significant differences in serum Cu were observed among Balb/c groups in *Leishmania major* infection. However, an increase in serum Cu of infected C57bl/6 mice was observed when compared to control mice of the same strain, Zinc deficiency in infected Balb/c mice was observed when compared to control Balb/c mice whereas no significant differences were observed in C57bl/6 mice. The Cu/Zn ratios were similar in the two groups of mice; there was a significant increase in infected Balb/c and C57bl/6 mice [22]. Davachi *et al*. (2009) observed statistically significant differences in serum Cu

among mice infected with *L. major*. Serum Cu decreased in test group compared to control group in uninfected Balb/c mice and Serum Zn increased in test groups as compared with control group in infected Balb/c mice groups. The ratio of Cu/Zn decreased, but this decline was not significant [5].

In the present study, serum Cu and Zn levels were compared after inhibition with DEX or induction with LPS in NMRI mice infected with rodent malaria. Results indicated statistically significant differences in serum Zn, Cu and Cu/Zn ratios. Zn decline and Cu increase in test group were observed in both DEX and LPS treatment. However significant increase of serum Cu/Zn ratio was observed only in DEX mice. Although, the results of this study indicated variations of Zn, Cu and Cu/Zn ratios in rodent malaria after inhibition with DEX or induction with LPS, however these microelements alterations are depending on several factors and may probably on several mediators and cytokines [22-24] which are required more studies to be clarified.

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